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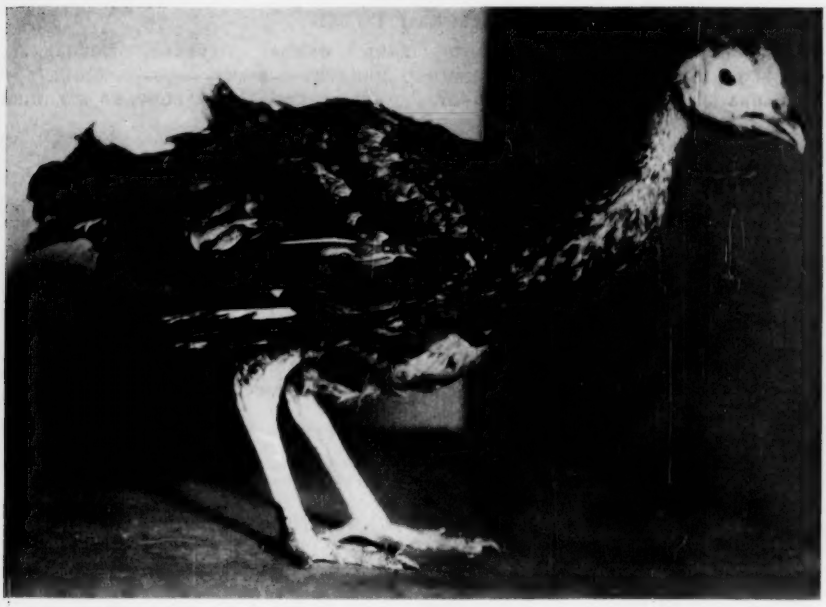

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Haploid Tobacco

Why Human Genetics?

Fetal Malformations in Swine

"Cancer Eye" and Harelipped Cattle

The Modification of Centromere Activity

VOLUME 51 MARCH-APRIL, 1960 NUMBER 2

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The JOURNAL OF HEREDITY is published bi-monthly by the AMERICAN GENETIC ASSOCIATION at 32nd Street and Elm Avenue, Baltimore 11, Md., and 1507 M St., N. W., Washington 5, D. C. Address all communications regarding editorial matters to 1507 M St., N. W., Washington, D. C. Communications regarding the business management of THE JOURNAL OF HEREDITY may be addressed to the publication office, 32nd Street and Elm Avenue, Baltimore, Md., or to 1507 M St., N. W., Washington, D. C. Entered as second-class matter February 24, 1923, at the postoffice at Baltimore, Maryland. Acceptance for mailing at the special rate of postage provided for in section 1103, Act of October 3, 1917, authorized December 29, 1918.

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$8.00 a Year. $2.00 a copy. Prices of back numbers on application.

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Journal of Heredity

Vol. LI **MARCH-APRIL, 1960** **No. 2**

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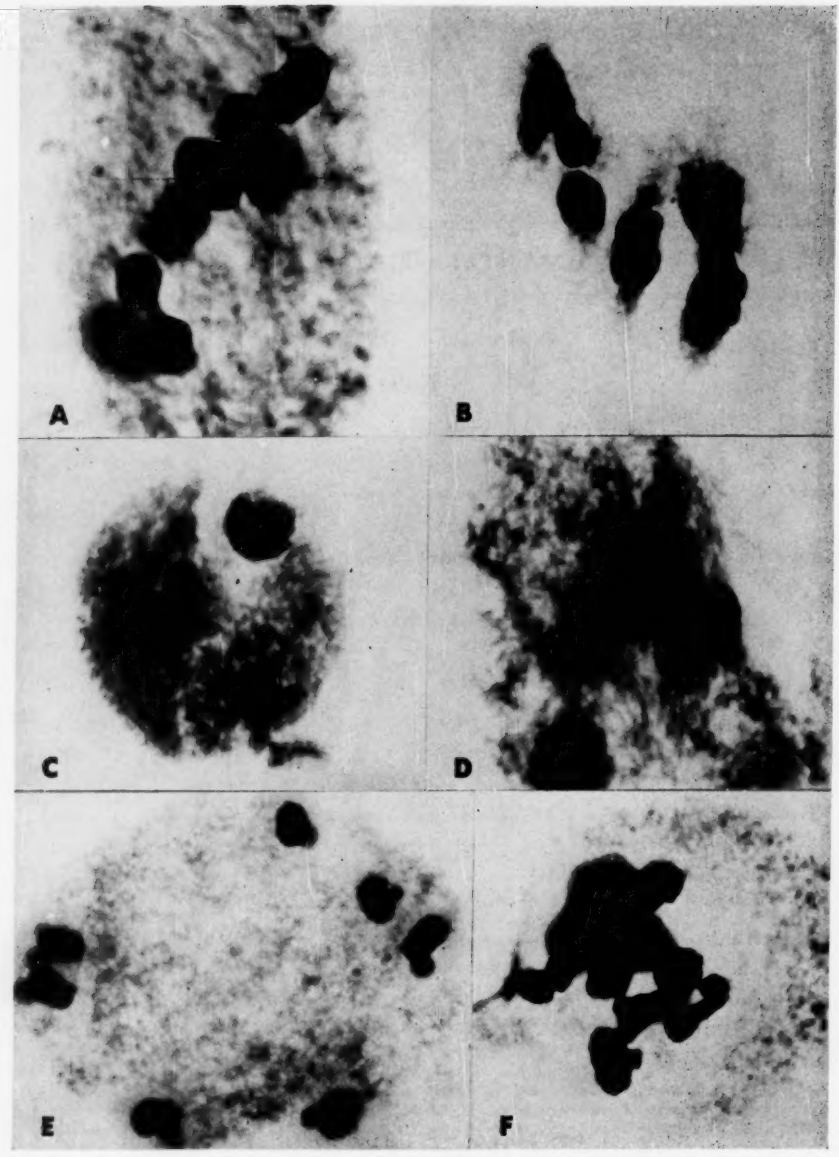


ERRORS IN SPINDLE AND NUCLEOLAR ORGANIZATION

Figure 1

A—Pollen-mother-cells showing an inclined metaphase plate (× 3240). *B*—An incompletely formed sipndle; the fiber elements associated separately with each of the bivalents (× 2025). *C* and *D*—Pollen-mother-cells showing compactly formed diminutive spindle with the bivalents crowded on the plate but with their centromeres co-orientated (× 1950, 2225). *E*—Diakinesis showing adventitious formation of nucleoli by all of the chromosomes (× 2520). *F*—Another cell showing abnormal and excessive formation of nucleolar material (× 1920).

EXPERIMENTAL MODIFICATION OF CENTROMERE ACTIVITY

H. K. JAIN*

WITH the end of prophase I in meiosis, bivalents take up a position on the metaphase plate in a characteristic manner, showing co-orientation of their centromeres. Most interpretations of this arrangement postulate a state of balance between various forces which can be inferred from the behavior of the chromosomes. During the course of some cytogenetic experiments on perennial rye grass which were planned primarily to study the effect of high temperature on crossing-over, it was found possible to induce modifications in the so-called metaphase equilibrium so that the bivalents assemble on the equatorial region without orientation of their centromeres. The present report deals with this failure, its interpretation and related questions.

Material and Methods

Plants of a clone of rye grass (*Lolium perenne* L.) which has been maintained at the Welsh Plant Breeding Station for a number of years formed the experimental material. These were subjected to temperature treatment of $31 \pm 2°C$ for a continuous period of 38 hours which was so timed that at the end of it, a large number of pollen-mother-cells in several of the spikes were showing meiotic division. The cells were thus exposed to high temperature during part of meiosis and during the preceding resting and pre-meiotic mitotic stages. The inflorescences were fixed immediately after the treatment, which was given by keeping the potted plants in electrically heated and thermostatically controlled heat chambers. The selected spikes were fixed and stored in Carnoy with a few drops of aq. ferric chloride solution (one percent) added to it. Aceto-carmine was used for making the squash preparations.

Results

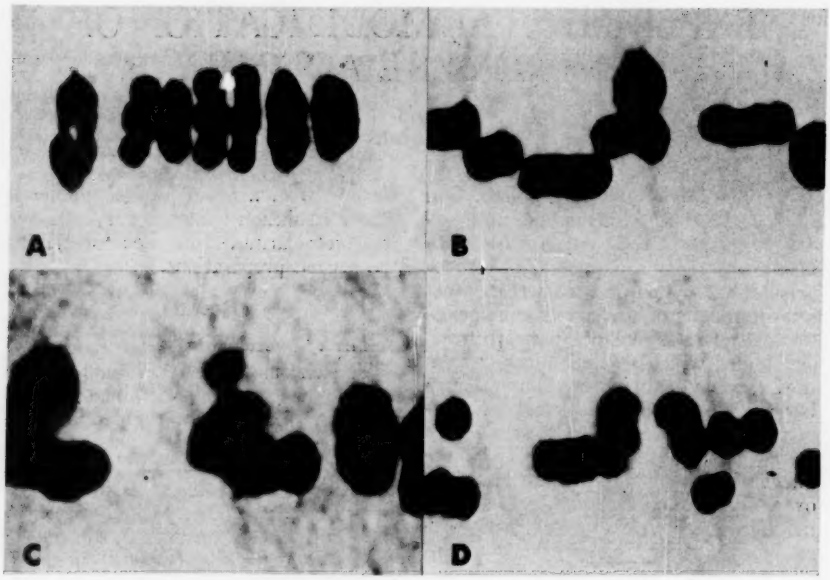
Failure of co-orientation

The chromosomes of *Lolium* have a well-defined centromere; the seven bivalents normally show regular arrangement and co-orientation on the metaphase plate (Figure 2*A*). The abnormal behavior of the centromeres in the temperature-treated material is indicated by their failure to be orientated towards the poles, although the bivalents assemble on the equatorial region quite regularly (Figure 2*B*). Associated with the irregular metaphase arrangement of the chromosomes is a noticeable lack of attenuation of the centromeric regions which further emphasizes the relatively inactive state of the centromeres in the treated material. In the absence of this attenuation the bivalents appear round in form and over-condensed.

Defects in spindle organization

Not all the pollen-mother-cells showing disturbed metaphase arrangement of the bivalents were suitably fixed and stained for observations on the spindle, but defects in the organization of this body could be observed in a large number of them. In these the spindle is found to be poorly formed with the fibers loosely arranged, giving it an incompact appearance (Figure 2*C* and *D*). In some of these cells the metaphase plate shows an inclined position (Figure 1*A*). The incompletely formed spindles are particularly evident in those

*Division of Botany, Indian Agricultural Research Institute, New Delhi. These experiments formed part of studies undertaken at the Department of Agricultural Botany, University College of Wales, Aberystwyth. I am grateful to Prof. P. T. Thomas for helpful suggestions, particularly during the writing of the manuscript. Thanks are also due to Mr. A. R. Beddows for his kind offer of the plants used in this study.



IRREGULAR METAPHASE ARRANGEMENT AND INCOMPACT SPINDLES
Figure 2

A—Pollen-mother-cells (control) showing regular arrangement of the bivalents with the centromeres co-orientated (× 2750). *B*—Irregular arrangement of the bivalents, several of which fail to have the centromeres properly orientated (× 2780). *C*—Incompactly formed spindle with the over-condensed bivalents assembled in two groups (× 3450). *D*—Univalents and bivalents loosely attached to an incompact spindle (× 2700).

cells in which each of the bivalents is found to be associated with its own fiber elements which are clearly too inadequate for the formation of a single compact figure of normal size (Figure 1*B*). The poor formation of the fiber elements is indicated in a different way in a few of the pollen-mother-cells. In these a compact and well-organized spindle is formed, but this is invariably of a small size occupying only a limited space in the cell with the bivalents very much overcrowded on it (Figure 1*C* and *D*). An interesting feature of these diminutive spindles is that the bivalents on them show normal orientation of their centromeres.

The failure of co-orientation described above occurs in a majority of the pollen-mother-cells. In others, the effect of temperature treatment takes a more extreme form. In these, spindle formation is apparently delayed or fails altogether, and the chromosomes remain scattered along the cell periphery in a random manner, without any suggestion of arrangement.

Formation of adventitious nucleoli

The disturbed metaphase arrangement of the chromosomes is preceded by stages which are quite normal except in relation to the organization of the nucleolus. Cytological analysis of these stages has not been as complete as that of metaphase, but it could be determined that unlike the control material in which one or two nucleoli are organized in the nucleus at particular sites, in the treated material individual chromosomes form their own nucleolar material which mostly remains associated with them (Figure 1*E* and *F*). The effect of temperature treatment in inactivating the nucleolar-organizing region which leads to the adventitious formation of nucleoli

by the various chromosomes has been described fully elsewhere, in relation to a different clone of *Lolium* subjected to similar experimental conditions[5].

Following the defective development of the spindle and the irregular metaphase arrangement, the chromosomes fail to show anaphase separation and eventually form restitution nuclei. No viable pollen grains are obtained.

Discussion

The establishment of the metaphase configuration constitutes one of the most important steps in the process of nuclear and cell division. The mechanism determining this arrangement of the chromosomes is difficult to analyze experimentally. Several theories have been put forward which seek to account both for the metaphase orientation of the chromosomes as well as for their anaphase separation on the basis of forces of attraction and repulsion. While the existence of such forces is indicated rather strikingly in some organisms, particularly in the spermatocytes of several insects[4, 7], it has to be recognized that no generalizations are permissible from the present evidence. In fact Hughes[3] has pointed out that the suggesting of these forces in many cases has nothing more than linguistic convenience to recommend it. An interesting feature of the present observations is that the failure of co-orientation is found to occur independently of the process of assembling, which remains mostly unaffected by the temperature treatment. This specificity of the response appears to be inconsistent with the hypothesis of Ostergren[6] who postulates that co-orientation and assembling are intimately correlated, being controlled by the same force of attraction from the poles. A second obviously important feature of the results is that apart from showing failure of orientation of the chromosomes, the affected pollen-mother-cells also show poor development of the spindle. This fact naturally suggests that the disturbed metaphase arrangement is related to the defective organization of the achromatic figure. The suggestion finds support in the hypothesis put forward by Swann[8], that the establishment of the metaphase configuration is determined by the forces of aggregation at work between the hydrated fibrillar systems combined in the spindle. This hypothesis of course applies to those spindles where orientated regions first form round the individual centromeres and later on coalesce to give the achromatic figure, as happens quite commonly in the case of plants. In the present material, as already described, the fiber elements generated around the individual bivalents may fail to fuse together and to form a single body. In others, they fuse imperfectly giving poorly developed spindles. The importance of the process of organization of the mitotic figure in determining the metaphase configuration has been emphasized in recent years by other authors also[9].

A possible explanation for defects in the organization of the spindle in the temperature-treated plants is suggested by the exceptional behavior observed in a few of the cells which have well-organized spindles although these are greatly reduced in size. These small spindles, as well as the non-formation of the achromatic figure in some other cells, indicate that the fiber elements produced in most of the cells are too inadequate to give a compact figure of normal size and form. A well-organized spindle is thus possible only at the expense of its size. The normal metaphase arrangement of the bivalents on these small spindles is also of great interest, for it is entirely consistent with the interpretation of the process of establishment of the metaphase plate discussed above.

The poorly developed spindles in the present material may be instructively compared with the irregular types observed by Darlington and Thomas[2] in a trisomic derivative of a cross between *Lolium* and *Festuca*. The two differ in the fact that in the treated *Lolium* plants the defective spindles are found to be associated with an almost total failure of co-orientation of bivalents. Such failure occurs much less commonly in the trisomic plant, in which abnormal behavior manifests itself more characteristically in irregular anaphase separation, because of the incompact nature of the spindles and results, as suggested by the authors, from a lack of co-ordination between the spindle-organizing activities of the centromeres and the external agents. Incompactness of the spindles in the present material is of a different nature, and this fact probably accounts for the failures observed to be different in the two cases.

It may be concluded that the failure of co-orientation of bivalents in the treated material results from defects in the organization of the spindle which in turn is determined by poor formation of the fiber elements. A possible explanation for the failure of the spindle fibers to be produced normally may now be considered. The present observations and those reported previously have shown, as pointed out earlier, that the high temperature treatment inactivates the regular nucleolar-organizing regions, and when this happens each of the pairs or a varying number of them form their own nucleoli. The earlier observations have also shown that the nucleolar-forming chromosomes, which alone undergo normal prophase condensation, fail to show regular metaphase arrangement, while the chromosomes which fail to synthesize the nucleolar material adventitiously and remain relatively uncondensed arrange themselves on the metaphase

plate in a far more regular manner. These observations thus indicate that the ability of the chromosomes to synthesize nucleolar material adventitiously is negatively correlated with the normal functioning of their centromeres. The mechanism underlying this inverse relation is not very obvious; it can, however, be suggested as a possibility that the temperature treatment in *Lolium* induces the centromeres to become associated with nucleolar synthesis at the expense of their normal activity. The role of the centromeres in organizing the spindles, particularly in plants, is now generally recognized, although it has not been subjected to precise experimental analysis. According to the hypothesis put forward by Anderson[1], the kinetochores elaborate polycationic substances which bring about formation of the spindle fibers.

The suggestion made here that the centromeres may become associated with nucleolar formation under abnormal conditions is, however, to be treated with reserve as there is no direct evidence for activity of this nature.

Summary

In *Lolium*, the normal metaphase arrangement of the chromosomes has been modified by high temperature treatment in such a way that the bivalents assemble more or less regularly without showing co-orientation of their centromeres. Evidence has been presented which leads to the conclusion that the disturbed arrangement is related to the defective organization of the spindles resulting from inadequate formation of the fiber elements. The interpretation of the results provides support for the mechanism suggested by Swann for the establishment of the metaphase configuration, and also for the conclusion that the temperature treatment probably induces the centromeres to become associated with adventitious nucleolar formation at the expense of their normal activity.

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STUDIES ON BOVINE OCULAR SQUAMOUS CARCINOMA ("CANCER EYE")

V. Genetic Aspects

DAVID E. ANDERSON*

OCULAR squamous carcinoma has a frequency in bovines several times higher than all combined types of malignant tumors in man, develops in a highly localized area of the body, and is influenced by circumocular pigmentation. The disease is similar to cancer in man and might provide clues having practical application, since control could result from knowledge of the relation between pigmentation and susceptibility and, in the bovine, from knowledge of the inheritance of these traits as well. Study of these objectives in the present case was not influenced by factors such as race, geography, cultural customs, socio-economic status, occupation, sex, age, and variability in diagnoses, which are sometimes involved in studies of this sort with human material.

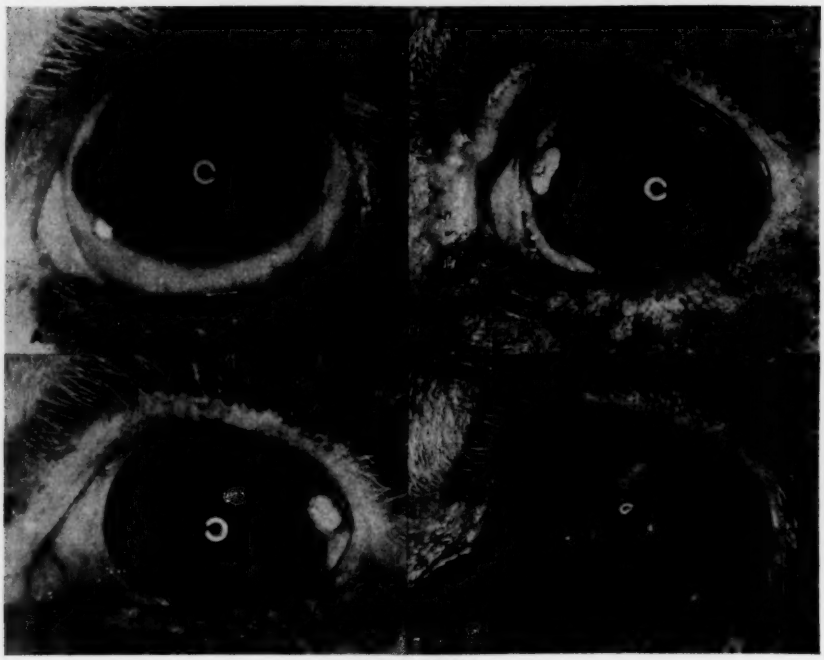
According to Russell *et al.*[15], the developmental pathology of bovine ocular squamous carcinoma is apparently a continuous process wherein lesions on the eyeball, nictitating membrane, and caruncle proceed from a plaque, sometimes through a papilloma stage, to carcinoma; and those on the circumocular skin proceed from areas of keratoses and/or acanthoses with focal ulceration, sometimes through a papilloma state, to carcinoma. Illustrations of some of these lesions are shown in Figure 3.

The disease increases in frequency with age. The lesions may be multiple in either eye and may involve one or more anatomic sites. Their most frequent site of development is the limbus of the eyeball. The mucocutaneous junction of the lower lids and that of the lacrimal lake are frequently involved, followed by the nictitating membrane and caruncle. The bulbar conjunctiva, mucocutaneous junction of the upper lids, and the cornea proper are involved only rarely. A more definitive study of the relative frequency of ocular squamous carcinoma and its benign precursor lesions will be forthcoming in a separate publication of the Cancer Eye Study Section.

Materials and Methods

This report is based on six routine observations of eyes made at six-month intervals on each of 105 Hereford cows at the Ft. Reno Research Station in El Reno, Oklahoma. The observations commenced in April 1954, when all the cows were six years of age, and continued to October 1956, when they were eight and one-half years of age. At each observation eyes were clinically examined and photographed. Some lesions (approximately 20 percent) were biopsied or excised *in toto* for histopathologic study and tissue culture. Some excisions were also to prolong the productive life of the affected animals. Moreover, each observation provided information on the anatomic site of origin of a lesion, the progression and regression, age of first occurrence, and number of involved sites. About 80 percent of the biopsies and excisions were at the last or next to the last

*Department of Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas. This is publication No. 5 of the Cancer Eye Study Section. The investigation was supported in part by Grant G-1 from American Cancer Society, Grant C-1751 and C-2890 from National Cancer Institute of the National Institutes of Health, Public Health Service. The author wishes to express his appreciation and gratitude to Dr. D. Chambers, Oklahoma State University, for his suggestions during the study, to Dr. J. L. Lush, Iowa State University, for his valuable criticisms and suggestions during the preparation of the manuscript, and to Dr. E. Staten Wynne, Department of Pathology, for the clinical and microscopic diagnoses of lesions.



EYE LESIONS
Figure 3

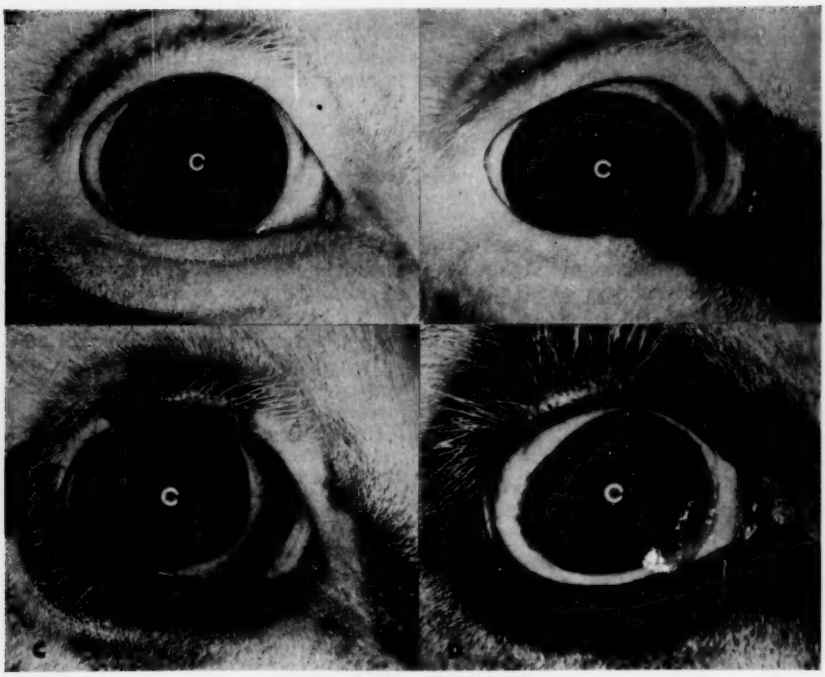
A—Plaque at medial limbus. One also at lateral limbus but not readily discernible in black and white photograph. *B*—Papilloma at medial limbus, also acanthosis with focal ulceration in area of lacrimal lake and medial canthus. *C*—Keratosis on upper and lower lids, acanthosis with focal ulceration at medial canthus, and early carcinoma at lateral limbus. *D*—Carcinoma at lateral limbus.

observation, and thus should not have materially influenced site frequency and progression and regression values.

Both ocular squamous carcinoma and its benign precursor lesions were classified for present purposes according to site of origin as follows: those developing on the circumocular skin including the upper and lower lids, lacrimal lake and caruncle were classified as "lid lesions." These sites are generally pigmented. Lesions developing on the eyeball and nictitating membrane were classified as "eyeball lesions," these areas being relatively unpigmented.

Information on pigmentation in the skin of the lids and hair of the circumocular region, which varies in shade from a yellowish-brown to an intense reddish-brown, was obtained from 2 × 2 colored transparencies of the 105 original animals and their progeny. Pigmentation was measured as an amount by counting the number of units covered by pigment on a scale which divided lid length into 100 equal units. The amounts ranged from zero percent for an eye completely devoid of pigment to 100 percent for one completely surrounded by pigment, and averaged about 30 percent for the right and left eyes. The amounts for both eyes were summed for a measure of total circumocular pigment. Examples of varying amounts of pigment are shown in Figure 4.

A more-or-less quantitative measure was constructed for measuring individual variability in susceptibility by considering the age at which squamous carcinoma or its benign precursor lesions



CIRCUMOCULAR PIGMENT
Figure 4

A has zero amount of pigment; *B* has 30 percent; *C* has 66 percent, and *D* is completely pigmented with 100 percent.

first developed, the number of sites on which lesions developed, and the number which regressed between successive observations during the 30-month observational period. Both the time required for tumors to develop (age) and number of nodules (which corresponds to number of sites) have been shown to measure adequately the variability in susceptibility to pulmonary tumors among groups of mice of different genotypes (Heston[9, 10]). Lombard *et al.*[12], Warren and Ehrenreich[18], and Warren and Gates[19] considered the occurrence of primary multiple tumors of the same or different organs in excess of that expected by chance alone to indicate increased susceptibility in some persons. Blackwell *et al.*[5] utilized differences in the age at which cattle, with and without eye tumors, were removed from a herd in order to develop a measure of susceptibility. The basic assumption was that susceptibility is a continuous variable. Thus, animals developing tumors early in life were regarded as more susceptible than those developing them later (in the same general environment); and older animals among the normal ones were regarded more resistant than the younger, since some of the young would probably have developed more tumors at older ages if they had remained in the herd.

The spontaneous regression of lesions to types of lesser significance (according to the classification of Russell *et al.*[15]), or spontaneous regression to clinically normal eyes, was also considered in the present case to be indicative of individual variability in susceptibility. An animal with a high number of such regressions was assumed to have a higher degree of resistance than one with a

smaller number of regressions, and this latter one in turn a higher degree than an animal whose lesions showed no change. Regression is used here to mean any abatement or diminutive change of a lesion between two successive observations, which may or may not have been followed by recurrence to the original stage of significance, or transformation to a higher one. More definitive information on regression will also be forthcoming in a separate publication of the Cancer Eye Study Section.

A different scheme was used in measuring variability in age of onset than that given by Blackwell *et al.*[5], although both methods were based on the same general assumptions. The age-range in Blackwell's study was from three to 15 years and it was assumed that the statistical distribution of susceptibility was "normal." Thus, their scoring system utilized the normal curve. In the present case, the use of a strictly linear score was indicated by the correlation of .99 between age and tumor frequency for ages from six to eight and one-half years. A value of 6 was assigned to an animal first developing a lesion at six years of age, a value of 5 for one at six and one-half years, 4 for one at seven, and so on to a value of 1 for an animal with a lesion first developing at eight and one-half years of age. Actual counts were used for the number of sites involved and regression frequency. The number of sites ranged from 0 to 14 (based on 20 arbitrary anatomic sites on the lids and eyeball) and the number of regressions ranged from 0 to 5. The score of susceptibility was had by simply adding the figures for age and number of sites and subtracting the number of spontaneous regressions. Thus, a high figure is meant to denote a high degree of susceptibility. The score was computed individually for lid susceptibility in the right and left eyes, in each of which the values ranged from 0 to 11 and averaged 1.7; and for eyeball lesions in each eye, where the values ranged from 0 to 9 and averaged 4.0. These values were summed for measures of lid, eyeball, or total susceptibility. The latter score was distributed nearly normally with a range from 0 to 31 and an average of 11. Normal animals received a score of zero. Only eight, or 7.6 percent of the animals, remained clinically normal throughout the entire 30-month observation period.

Results

The phenotypic relationship between circumocular pigmentation and lid lesions, without regard to those on the eyeball, is shown in Table I. The number of animals with lesions and amounts of pigment less than 21 percent exceeds the number expected if lesions and pigment were independent. As the average amount of pigmentation increases above 20 percent, the observed number with lesions is correspondingly less than expected. No lid lesions were observed among 18 animals with amounts in excess of 80 percent although eight animals were expected to have such lesions. The probability is much less than .001 that the excess of lesions in non-pigmented animals, and the deficit of lesions in pigmented ones, could have occurred purely by chance if these lesions were really independent of pigment. This is in keeping with the relationship reported by Anderson *et al.*[3] in a study involving 338 affected animals among a total of 842, including the present animals. Bonsma[6], Guilbert *et al.*[7], Lush[13], and Wernicke[20] also noted more lesions among non-pigmented lids than pigmented ones. Their findings, however, were

TABLE I. Observed and expected frequencies of ocular squamous carcinoma and its benign precursor lesions in animals with varying average amounts of circumocular pigmentation

Condition of lids		Percent pigmentation						Total	Chi square
		0	1-20	21-40	41-60	61-80	81-100		
Lid lesions	obs.	14	17	9	7	3	0	50	
	exp.	8.5	10.0	10.5	7.5	5.0	8.5		34.3**
Normal lids	obs.	4	4	13	9	7	18		
	exp.	9.5	11.0	11.5	8.5	5.0	9.5		
Total		18	21	22	16	10	18	105	

**$P < .001$.

based on relatively few affected animals (33 out of some 4,500).

Correlations between amount of circumocular pigmentation (on a quantitative scale from 0 to 100) and the score of lid susceptibility were —.48 for the right eyes and —.43 for the left eyes, with an average of —.46 ±.08. The latter is a measure of the closeness of the relationship shown in Table I. The negative signs show that high lid susceptibility occurred in eyes having small amounts of pigment and low susceptibility in eyes having larger amounts. One could imagine either that circumocular pigment inhibits lid lesions directly within the eye in which pigment occurs, or that the effect of pigment is a more generalized one caused by pleiotropy far back in development. That the former may be more nearly correct was shown by partial correlations, i.e., between right pigment and left susceptibility in groups having similar amounts of pigment in the left eye, and vice versa. The two resulting correlations averaged —.07 ±.10, which is not significantly different from zero. Using the same technique for the correlations between pigment and susceptibility within the same eye in groups having similar amounts of pigment in the other, an average correlation of —.36 ±.09 was obtained. Since the partial correlations between pigmentation in one eye and susceptibility in the other were essentially zero, while the ones between pigmentation and susceptibility in the same eye were only reduced slightly from their first-order value of —.46, the indication is that circumocular pigment affects lid susceptibility directly, presumably by shielding the area of the lid in which it occurs from some environmental carcinogen, such as solar radiation. This effect is evident in Table I where lid lesions are not observed in the last pigmentation class. Furthermore, lesions were never observed in any pigmented area of the lids, regardless of the shade of pigment. Lesions did develop in non-pigmented areas of partially pigmented lids but not so frequently as on lids with less pigment. The absence of a generalized inhibitory effect was also shown by the correlations between pigmentation and eyeball susceptibility which averaged —.12 ±.10 whether they were between these traits in the same eye or in opposite eyes. The inhibitory effect of pigment, therefore, is seemingly a direct one confined only to lid susceptibility, and not extending beyond the pigmented areas themselves.

Hereditary Variations in Susceptibility

The variation within pairs of eyes (i.e., between the right and left eyes of the same animal) is presumably wholly non-genetic, consisting of developmental errors during the embryological period, non-linearity between the score of susceptibility and primary gene effects, and environmental effects. The variation between pairs of eyes contains the additive and non-additive genetic and all the environmental variance which is caused by environmental events common to both eyes. Estimates of heritability can be computed from components of variance between and within pairs of eyes, but these will be maximal estimates for they involve the genotype as a whole besides any environmental and genotypic-environmental components which are alike for both eyes. These maximal estimates may be compared to conventional ones, which contain little except additive genetic variance to throw light on the nature of the observed variation in pigmentation and susceptibility. Table II shows the components of variance for pigmentation and susceptibility and the resulting intraclass correlations or maximal estimates of heritability. The approximate 95 percent confidence intervals are also shown.

The estimate of .56 for circumocular pigmentation is slightly larger than a conventional heritability estimate of .45 reported by Anderson *et al.*[4], in a study including the present animals along with others. This relatively small difference could mean that interactions between allelic and non-allelic gene pairs (dominance and epistasis) and environmental differences common to both eyes have small effects on amount of circumocular pigment. A

TABLE II. Components of variance for circumocular pigmentation and lid, eyeball, and total susceptibility for 105 eight and one-half year old animals

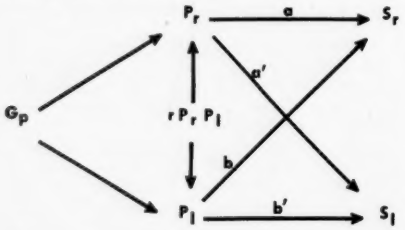
Source of variation	Degrees of freedom†	Expected mean squares	Components of variance: Lid sus.	Eyeball sus.	Total sus.	Pigmentation
Total	208					
Between pairs of eyes (b)	104	$\sigma w^2 + 2\sigma b^2$	4.929**	2.970**	8.862**	748.453**
Within pairs of eyes (w)	104	σw^2	2.519	4.938	10.404	577.077
Intraclass correlations			.66	.38	.46	.56
95% confidence intervals			.50-.8	.22-.54	.30-.62	.40-.72

**P < .01.

†One animal with one eye, thus 105 animals but 209 eyes.

comparison of these two estimates suggests that about 45 percent of the phenotypic variability is due to differences in additive genes, 11 percent (56 minus 45 percent) to differences in allelic and non-allelic interactions and environmental effects common to both eyes, and the remaining 44 percent, which is the variation between eyes on the same animal, largely to non-genetic factors which would cause amount of pigmentation in one eye to differ from the other.

The estimates for lid, eyeball, and total susceptibility were .66, .38, and .46, respectively. The one of .46 for total susceptibility compares favorably to a conventional heritability estimate of .41 for total susceptibility reported by Anderson *et al.*[2]. However, the high estimate of .66 is suspect. Rather than measuring the concordance of lid susceptibility in both eyes, it might be an indirect measure of the concordance of pigment because pigmentation and lid susceptibility in the same eye are highly related, as is the amount of pigmentation in both eyes. The extent to which the correlation of .66 may have been influenced by pigmentation was investigated by a path coefficient analysis as outlined by Wright[21]. The biological diagram in which pigmentation is assumed to be the sole cause of variation in lid susceptibility is as follows:



where a, a', b and b' are path coefficients (standardized partial regression coefficients), P and S refer to pigmentation and susceptibility, rP_rPl refers to the correlation between pigmentation in the right and left eyes, and G_p to genes determining pigmentation. The expected correlation between lid susceptibility in the right and left eyes, if pigmentation were the sole factor in susceptibility, is the sum of the products of the path coefficients along all paths by which S_r and Sl are connected as follows:

$$aa' + bb' + a'brP_rPl + ab'rP_rPl \quad (1).$$

Solving for the four unknown path coefficients knowing that $rP_rP_l = .56$, and then substituting these into equation (1), an expected correlation of .16 is obtained. Thus, the correlation between lid susceptibility in the right and left eyes, after subtracting the influence of pigmentation being similar in the two eyes, would be approximately .66 minus .16 or .50. The correlation for eyeball susceptibility was not corrected for pigmentation because the two were not significantly related. The correlations in Table II show that pairs of eyes are obviously more alike in their susceptibilities (even for lid susceptibility) than is explainable by pigmentation alone. Bilaterality of lesions will be discussed in a separate publication of the Cancer Eye Study Section.

The estimates for lid and eyeball susceptibility would be expected to be similar if lesions at these sites were expressions of the same disease with different anatomic sites of origin. The corrected estimate for lid susceptibility of .50 and the one of .38 for eyeball susceptibility compared to the conventional estimate of .41 suggests that about 40 percent of the phenotypic variability in lid susceptibility is due to differences in additive genes, while about 10 percent is due to differences in allelic and non-allelic interactions, environmental factors common to both eyes, and interactions between genotype and environment. However, the excess of .50 over .38, in view of their expected similarity and the similarity between the eyeball and conventional estimate, could mean that allelic and non-allelic interactions have little effect on lid susceptibility. The 10 percent for lid susceptibility would then be due largely to differences in environmental factors and their interactions with genotype influencing the lids of both eyes over and above the additive genetic influence on susceptibility and pigmentation. The remaining 34 percent could be ascribed to differences in non-genetic factors which differ between eyes on the same animal or to incompleteness in the measure of susceptibility. It seems unlikely that sampling variation was responsible for the difference between the estimates for the lids and eyeball, although their approximate 95 percent intervals overlap, because a similar difference in the same direction was observed in another herd[1]. Further indications that environmental factors may have more influence on the lids than the eyeball are: (1) lid lesions develop at a slightly earlier average age than those on the eyeball, (2) they are more frequent on the more exposed lower lids and lacrimal lake than the upper lids even though the lower lids have a higher average amount of pigment, and (3) lid lesions do not develop in pigmented areas of the lids.

That the maximal estimate of .38 for eyeball susceptibility is similar to the conventional heritability estimate of .41 suggests that about 40 percent of the variability in eyeball susceptibility is due to differences in additive genes, and the remaining 60 percent to differences in factors which differ between eyes.

Discussion

The results just presented show circumocular pigmentation to have a direct influence on lid susceptibility to ocular squamous carcinoma by inhibiting the appearance of lesions in pigmented areas of the lids. The high concordance between eyes on the same animal

points to factors having correlated effects on the susceptibilities of both eyes over and above the effect of pigmentation. Genetic factors appear to account for most of these correlated effects to the extent that about 40 percent of the phenotypic variation in lid and eyeball susceptibility is due to additive genetic differences. Differences due to allelic and non-allelic gene interactions seem to have little influence on susceptibility although environmental factors and their interactions with genotype have about 10 percent more influence on the variability of lid than eyeball susceptibility. Additive genetic differences appear responsible for about 45 percent of the variation in pigmentation, while both additive and non-additive differences account for about 56 percent.

That lid pigmentation is heritable, coupled with the finding that pigment has a direct inhibitory effect on lid lesions, implies that genes for pigment also influence lid susceptibility. Moreover, that this direct inhibitory effect of pigment is manifest by the absence of a relationship between pigment in one eye and susceptibility (either lid or eyeball) in the opposite eye and by presence of lesions in non-pigmented areas of partially pigmented lids is suggestive that gene *loci* for pigment and those for susceptibility are independent. Otherwise, pigment would have shown more generalized effects on susceptibility extending to areas in the ocular region beyond the pigmented areas themselves. The relation between pigmentation and susceptibility might be summarized as one wherein the gene *loci* for these traits are independent, and *loci* for susceptibility influence this trait; but those for pigment also influence lid susceptibility by their intermediate effect on pigment.

The present findings are somewhat similar to those reported by Steiner[16, 17] for negroids where the relative lack of skin cancer was also explained by the degree of pigmentation of the skin. Albino negroids, however, were susceptible to actinic cancer. Pigmentation was the factor inherited, and not resistance of the cells to neoplastic transformation following solar radiation. Genetic factors for resistance to skin cancer in the negroid might also be involved but are not manifest because the pigmented skin protects against solar radiation. A similar situation might prevail in pigmented breeds of cattle. These rarely manifest skin cancer, but when they do, it is in unpigmented areas of the skin. This has been observed to occur in Friesians and Shorthorns (personal observation and Rice[14]).

Pigmentation seems to be involved in skin cancer in caucasoids, although to a lesser degree than in negroids, because individuals with light complexions show more predisposition to skin cancer than those with darker complexions. Hall[8] reported a study of 100 consecutive cases of microscopically proved skin cancer in which 87 percent of the cases were observed in light-eyed persons and 13 percent were in brown-eyed persons. The clinic population consisted of 63 percent brown-eyed and 37 percent light-eyed persons. Genetic factors for susceptibility to skin cancer, independent of those for pigmentation, are indicated by studies showing a multiple occurrence of tumors in some individuals. Lombard *et al.*[12] found that individuals with skin cancer had significantly more other cancers of the skin than expected by chance alone. Warren and Gates[19] also found for patients with primary skin cancer, over twice the expected rate for cancer of organs other than skin. Although multiplicity of tumors constitutes strong evidence for the important role of heredity in susceptibility, it could be strong evidence for the important role of some other etiological factor in skin cancer. The manifestation of susceptibility to skin cancer in man, ocular squamous carcinoma in the bovine, and other tumors is seemingly dependent upon whether or not the combination of genetic and environmental factors falls below or surpasses a physiological threshold as suggested by Heston[11]. If the genotype places an individual near the threshold, the probability is high that environmental factors would push it above the threshold. If the individual is not highly susceptible genetically, the probability is small that environmental factors would push it above the threshold early in life, but increases as the individual becomes older. The presence of skin pigment apparently prevents or at least hinders this threshold from being reached in actinic skin cancer. These relations suggest that if pigmentation could be augmented by chemical means, or if exposed surfaces of the body were merely protected, the probability of skin cancer in man from solar radiation should be considerably lessened.

Control of bovine ocular squamous carcinoma also seems possible because of the high relationship between amount of circumocular pigmentation and susceptibility to lid lesions, and the relatively high heritabilities of susceptibility and pigmentation. Phenotypic selection for an increased amount of circumocular pigmentation in both eyes would reduce the frequency of lid lesions. This procedure would have little, if any, effect in decreasing the frequency of lesions on the eyeball or nictitating membrane because of the lack of association between them and lid pigmentation. Furthermore, it is unlikely that such selection would reduce appreciably the total frequency of lesions because lid lesions are not as frequent as those on the eyeball, being 38 percent less frequent in the present study. Phenotypic selection for lid pigmentation alone (without regard to resistance) might bring about an increase in eyeball tumors. For instance, selection for pigment could conceivably result in a situation where lesions are "forced" to develop on the eyeball and nictitans, if they are to develop at all, because large amounts of lid pigment would hinder them from developing on the lids.

A reduction in total frequency of tumors could be accomplished by phenotypic selection for resistance. The heritability of susceptibility of .41 seems to make certain that this practice would be effective. In areas of high solar radiation, or in herds shipping animals to such areas, lid pigmentation could be a criterion for some selection at an early age, with more intense selection for resistance occurring later after an animal's resistance or susceptibility is more certain. Since age is an important factor in ocular squamous carcinoma, animals would need to be retained to eight or more years of age to be at all sure of their resistance or susceptibility. This procedure might be accelerated by family selection and possibly by use of a quantitative score of susceptibility. Such a score should increase the sensitivity of the measure of susceptibility over a simple all-or-none classification. This would be important particularly in herds with a low incidence rate. For example, in a herd with a rate of 50 percent, progress in selection would be almost doubled by a score; whereas in a herd with a rate of only five percent, selection progress would be quadrupled.

Summary

The relationship between amount of circumocular pigmentation and susceptibility to ocular squamous carcinoma and its benign precursor lesions was investigated in a study involving six observations on each of 105 cows between six years and eight and one-half years of age. Circumocular pigment was found to inhibit completely the development of lid lesions in pigmented areas of the lids. Lesions did develop in non-pigmented areas of partially pigmented lids but not so frequently as on lids with less pigment. These results were construed to indicate that gene *loci* for pigmentation and susceptibility are independent, but that genes for pigment also influence susceptibility by an intermediate effect on pigment. Both pigmentation and susceptibility exhibited genetic variability.

Lid susceptibility appeared to be influenced more by environmental factors than eyeball susceptibility. Solar radiation as an etiological factor particularly in lid lesions is suggested. Measures for controlling skin cancer in man and ocular squamous carcinoma in bovines are discussed.

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GENETIC PARAMETERS OF GLAND AND BODY WEIGHTS

In White Rock Cockerels

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ENDOCRINE glands are coordinators of body functions. The influence of the thyroids on basal metabolism, the role of the adrenals in adaptation and mineral metabolism, and the effects of the gonads in reproduction are well documented. However, little is known of the influence of heredity on the ductless glands or the genetic relationships between them.

While many estimates of genetic parameters pertaining to body weight *per se* in chickens have been reported[6], information on the association between endocrine glands and body weight is meager.

From full-sib correlations, El-Ibiary and Shaffner[3] reported heritability estimates of .44 and .29, respectively, for thyroid and testes weight in 72-day-old New Hampshires. Shaklee and Knox[11] computed heritability estimates of .92 and .77 for thyroid weight at four weeks of age in New Hampshire chicks. The higher estimate was based on full-sib correlations while the lower was obtained from a single generation selection experiment.

Using diallel matings, Jaap[8] obtained heritability estimates for bursa weight of .32 at hatching and .48 at 11 days of age in White Gold males. Phenotypic correlations between bursa and body weights were .47 and .79 at these ages.

El-Ibiary and Shaffner[3] calculated phenotypic, genetic, and environmental correlations between body and thyroid weights of .62, .52, and .70; between body and testes weights of .33, .13, and .40; and between thyroid and testes weights of .43, .40, and .46, respectively. A phenotypic correlation of .59 between body and thyroid weights was reported by Shaklee and Knox[11]. The correlations cited were based on actual gland weights unadjusted for body weight.

The investigation reported here was conducted to determine heritability estimates of, and the genetic, phenotypic, and environmental correlations between, 62-day-old body, adrenal, thyroid, testes, and bursa weights in non-inbred White Rock cockerels.

Methods and Materials

Data were obtained from two hatches of White Rock cockerels hatched two weeks apart. Birds from each hatch were fed the same ration and over-all management was similar.

At 62 days of age, the males were weighed to the nearest gram and sacrificed by bleeding. The bursa, thyroids, testes, and adrenals were removed and weighed immediately to the nearest 0.1 mg. Left and right testes and thyroids were pooled and weighed. Adrenal glands were weighed separately and analyses made for left, right, and total gland weights. All gland weights were adjusted to 100 grams of body weight.

Estimates of heritability, as well as genetic, environmental, and phenotypic correlations were computed from sire and dam components of variance and covariance according to standard methods[7, 9, 10]. Heritability estimates were calculated for both adjusted and unadjusted weights, while correlations were computed for the adjusted weights only. The statistical model used for the analysis of variance was:

$$X_{hijk} = \mu + a_h + s_{hi} + d_{hij} + e_{hijk};$$

where X_{hijk} was the record of the k^{th} progeny of the j^{th} dam mated to the i^{th} sire within the h^{th} hatch.

Analyses were based on 256 progeny

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from 109 dam families within 50 sire families.

Results

Hatch effects

Presented in Table I are means and standard deviations for measurements obtained in each of the two hatches. Despite the similarities in management, highly significant hatch effects were present for all traits except body and thyroid weights.

Heritability estimates

Heritability estimates based on full-sib correlations for values adjusted and unadjusted for body weight are presented in Table II. Estimates obtained from both sets of data are in close agreement. The heritabilities of body weight and adrenal weight may be considered high (>.40) while those for bursa weight and thyroid weight were of moderate magnitude (.24-.40).

The negative heritability estimates obtained for testes weights indicate that the genetic portion of the total variation for this character is very low at this particular age. Because of the negative variance components obtained for this trait, correlations between it and other characteristics would be of dubious value and were not calculated.

Correlations

Genetic, phenotypic and environmental correlations between the traits measured are presented in Table III. The three correlations between body and bursa weights were slightly positive and of similar magnitude. The phenotypic correlation between body and thyroid weights was essentially zero. This was due to the cancelling effect of the —.33 genetic and .26 environmental correlations. Of those traits measured, adrenal gland weight appeared to be the most highly associated with body weight. This association was negative. The genetic correlation between right adrenal weight and body weight was more than twice that between left adrenal weight and body weight. Contrariwise, the environmental relationship between body weight and left adrenal weight was higher than that between body and right adrenal weights.

Correlations between bursa weights and other gland weights, although consistently negative, were of a low order. It may be noted that in all comparisons the genetic association was higher than the environmental one.

Although the phenotypic correlations between adrenal and thyroid weights were close to zero, the genetic correlations were consistently negative and of moderate magnitude. The environmental correlations of .30 between thyroid and

TABLE II. Heritability* estimates adjusted and unadjusted for body weight

Trait	Adjusted	Unadjusted
Body weight		.63
Thyroid	.33	.24
L. adrenal	.42	.51
R. adrenal	.53	.51
Total adrenal	.57	.68
Bursa	.33	.36
Testes	—.18	—.18

$^{*}h^2 = \frac{2\,(S+D)}{S+D+E}$

TABLE I. Means and standard deviations by hatch for adjusted† values

Trait	Hatch 1	Hatch 2	Diff. (Hatch 1-Hatch 2)
Body weight (gm.)	1025.5±141.6	1017.4±121.9	8.1
Thyroid (mg.)	5.11±1.28	5.25±1.42	—.14
L. adrenal (mg.)	4.46±1.01	4.85±1.11	—.39**
R. adrenal (mg.)	4.95±1.03	5.48±1.14	—.53**
Total adrenal (mg.)	9.41±1.82	10.33±2.01	—.92**
Bursa (gm.)	.0609±.0167	.0687±.0274	—.0078**
Testes (gm.)	.0324±.0331	.0234±.0087	.0090**

**$p \leq .01$.

†Wt. /100 gm. body wt.

right adrenal weights, and .20 between thyroid and total adrenal weights, indicate a positive environmental association between these gland weights.

There were highly positive genetic and phenotypic correlations between left and right adrenal weights; however, the environmental association between them was considerably lower. As one might expect when a part is correlated to the whole, the correlations of left and right adrenals to total adrenal weight were very high.

Discussion

A highly significant hatch effect on bursa, testes and adrenal weights, even when uniform conditions were maintained from hatch to hatch, was shown in this study. While the differences between hatches for body and thyroid weights were not significant, this may not necessarily be consistent from trial to trial. Shaklee and Knox[11] obtained a highly significant hatch effect for thyroid weight in four-week-old chicks, but El-Ibiary and Shaffner[2] reported no hatch effect for this trait in birds at 10 weeks of age.

The heritability estimate of .63 obtained for body weight is higher than the average estimate of .30 reported for young chickens by Goodman and Godfrey[6], but is consistent with previous estimates obtained from the population under study[14]. The estimates of heritability of .33 and .44 for thyroid weight are similar to those reported by El-Ibiary and Shaffner[3] but considerably lower than those computed by Shaklee and Knox[11]. Heritabilities for bursa weights obtained in this study were within the range of those previously reported by Jaap[8].

Heritability estimates for left, right, and total adrenal weights ranged from .42 to .68, indicating that a large portion of the total variation is genetic. Garren and Shaffner[4] have reported that birds which seemed most resistant to muscular fatigue had the heaviest adrenals. If adrenal weight is related to function, selection of lines on the basis of adrenal size may be applicable in improving the fitness of the species.

The association between bursa and body weights observed in this experiment was considerably lower than those reported by Jaap[8] for males at hatching and 11 days. This would be expected because of the older age of the birds in the present study. Glick[5] found that bursa growth in Rhode Island Reds was greatest during the first three weeks after hatching but started to regress during the period from eight to 14 weeks. At nine weeks of age body weight would be increasing as bursa growth was declining and atrophy beginning. Thus, at this age, the correlation between these weights should be of a low order.

The phenotypic correlation between body and thyroid weights obtained in this study was essentially zero, whereas the genetic and environmental ones were of moderate magnitude but of opposite signs. These results differ considerably from those of El-Ibiary and Shaffner[3] who computed highly positive genetic, phenotypic, and environmental correlations be-

TABLE III. Genetic,* phenotypic† and environmental‡ correlations between traits for adjusted values

Traits	r	Bursa	Thyroid	L. adrenal	R. adrenal	Total adr.
Body weight	GG	.18	—.33	—.22	—.49	—.37
	PP	.12	—.02	—.32	—.41	—.41
	EE	.07	.26	—.45	—.30	—.47
Bursa weight	GG		—.09	—.11	—.24	—.18
	PP		—.04	—.06	—.13	—.11
	EE		—.02	—.03	—.06	—.05
Thyroid weight	GG			—.07	—.36	—.25
	PP			—.04	.02	—.00
	EE			—.01	.30	.20
L. adrenal	GG				.94	.99
	PP				.62	.89
	EE				.34	.82
R. adrenal	GG					.99
	PP					.90
	EE					.79

$$^{*}r_{GG} = \frac{2(S_{AB}+D_{AB})}{2(S_A+D_A)\ 2(S_B+D_B)}$$

$$^{\dagger}r_{PP} = \frac{E_{AB}+S_{AB}+D_{AB}}{(E_A+S_A+D_A)\ (E_B+S_B+D_B)}$$

$$^{\ddagger}r_{EE} = \frac{E_{AB}-S_{AB}-D_{AB}}{(E_A-S_A-D_A)\ (E_B-S_B-D_B)}$$

tween these weights. The methods of analyses in the two studies differed in that hatch variances and covariances were not removed in the analysis used by El-Ibiary and Shaffner and they did not adjust gland weights for body weight. It seems doubtful, however, that these differences would have such a marked effect on their results. Another reason for the differences in magnitude and sign of the correlations could be differences in the two populations. It may be noted that Siegel[13] computed genetic, phenotypic, and environmental correlations of −.34, −.11, and .23, respectively, between 59-day body and thyroid weights for this population, which are in close agreement with those obtained in this experiment.

The low order negative correlations between bursa weight and the other gland weights indicate, at best, a minor relationship between these traits at this age.

As would be expected, there was a highly positive genetic correlation of .94 between left and right adrenal weights. However, the environmental correlation of .34 was considerably lower than would be expected if both glands responded similarly to pituitary tropins. Bilateral differences between adrenal glands of chickens have been previously reported by Siegel[12] who showed that the right adrenal was significantly heavier than the left. The low phenotypic correlations between thyroid and adrenal weights were due to the cancelling effect of the negative genetic and positive environmental correlations between these traits. Although the growth curves of these glands are quite different, as demonstrated by Breneman[1], both glands are under the influence of the pituitary tropic hormones. It is possible that environmental factors stimulating the pituitary may result in similar responses from the right adrenal and thyroid glands. The positive environmental correlation between thyroid and right adrenal weight tends to support this reasoning.

The adrenal weight was more highly associated with body weight than was that of any other gland measured. Correlations between these traits were negative environmentally, phenotypically, and genetically. This would indicate that random environmental effects which have a positive effect on body weight have a negative effect on adrenal weight. Furthermore, genetic influences which tend to increase body weight cause a reduction in adrenal weight. In view of the cardinal role played by the adrenal in adaptive processes, it is probable that artificial selection for body weight would be counter to the aims of natural selection.

Heritabilities of body, bursa, thyroid, and adrenal weights were of sufficient magnitude at 62 days of age to indicate that mass selection for any of these characteristics should be effective in changing the population mean. All genetic correlations among these factors except the one between bursa and body weight were negative. These negative genetic relationships could be part of the method in which a population counteracts artificial selection.

Summary

Quantitative estimates were made of the interrelationships among body, thyroid, adrenal, and bursa weights in 62-day-old White Rock cockerels. Variation and covariation were subdivided into genetic and non-genetic proportions. The heritability of body weight and of adrenal weight was high (>.40) while that of bursa weight and thyroid weight was of moderate size (.24-.40).

Interrelationships between the traits measured were estimated by genetic, phenotypic, and environmental correlations. The possible influences of heredity and environment on the association between these characteristics are considered.

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THE TRANSMISSION OF THE MONOSOMIC CONDITION IN WHEAT

Var. Chinese Spring

KOICHIRO TSUNEWAKI AND E. G. HEYNE*

RESULTS of cytological studies on the transmission of the monosomic condition in Chinese Spring, a variety of common wheat, are important from several viewpoints.

The frequencies of the monosomics in progenies of monosomic parents must be determined by specific behavior of each chromosome during gametogenesis, maturation of the gametophyte and fertilization. Study of the transmission efficiency of the individual monosomic chromosomes can give some information on this point.

Monosomic analysis is an effective way to associate genes with specific chromosomes in polyploid species. A monosomic series of Chinese Spring is being used for this purpose in common wheat. For a critical analysis, it is important to know the actual frequency of the monosomics obtained on selfing the monosomic F_1 plant, since a hemizygous plant might be phenotypically different from a homozygous one.

A study of the radiosensitivities of monosomics and normal plants was made[14] which required knowing the frequency of occurrence for each monosomic. In studies at Kansas State University, monosomic and normal plants were classified by counting the chromosome number in somatic root tip cells. This method had not been used extensively, as a comparison of this method of analysis with other reported procedures was desired and is reported on in this paper.

Review of Literature

A series of isogenic monosomics was established by Clausen and Cameron[2] in *Nicotiana tabacum* and by Sears[12] in *Triticum aestivum* var. Chinese Spring. Monosomic series in other varieties of *T. aestivum* are being derived from Sears' monosomics by many workers. The isogenic monosomics were originally obtained from: 1) continuous backcrosses of a hybrid between amphidiploid and parental diploid species with the diploid parent[2, 5]; 2) crosses of haploids with the parental diploid[11]; and 3) asynaptic aneuploids which appeared in the process of 1 and 2[1, 13].

So far as meiosis of wheat is concerned, bivalents line up at the equatorial plate earlier than univalents[4], which split after the bivalents separate and move toward the poles. Such a gap in time between the divisions of bivalents and univalents causes frequent exclusion of the univalents from the daughter nuclei. The frequency of exclusion of monosomic univalents, therefore, can be estimated from the number and frequency of micronuclei formed in meiocytes. Morrison and Unrau[7] carried out an experiment to find the frequency of micronuclei in pollen tetrads of 20 different monosomics of wheat. They found that there was little fluctuation of frequency from spike to spike in the same plant or from plant to plant in the same kind of monosomic, but there were remarkable differences among different monosomics ranging from 34.1 to 65.2 percent. To the contrary, Olmo's work[10] with *N. tabacum* indicated that there was little difference in the percentage of univalent elimination in four different monosomics, that is 75.0 percent for the lowest and 76.8 percent for the highest frequency.

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The actual frequency of chromosome-deficient gametophytes can be obtained by cytological observations of pollen mitosis. Morrison[6] scored the number of pollen grains with 20 and 21 chromosomes for all of the monosomics of wheat except monosomic 14. Monosomic 21 showed the highest frequency of chromosome-deficient pollen grains with 96.3 percent and monosomic 7 showed the lowest with 11.1 percent.

The transmission rate of the monosomic condition through female gametes can be determined by crossing specific monosomics with normal pollen parents. The contribution of the male gamete to the transmission of univalents can be tested by crossing monosomic male parents to normal females. In *Triticum*, Nishiyama[8] reported that 71 to 73 percent of the eggs fertilized were chromosome-deficient, but only 11 to 37 percent of the functional pollen grains were deficient. Sears[12] estimated that the average frequency of functional eggs with 20 chromosomes was 75 percent and that of the pollen was four percent. Olmo[10] studied the same points in *N. tabacum* and found that the transmission of the monosomic condition through the egg showed a range of 45.6 percent for Haplo-N to 83.2 percent for Haplo-A, but the transmission through pollen was from zero for Haplo-C to 7.3 percent for Haplo-F.

Concerning a factor that affects the transmission of univalent chromosomes, Greenleaf[3] observed the development of female gametophytes and reached the conclusion that the slower rate of development and the higher frequency of abortion of (n-1) embryo-sacs were responsible for a lower transmission rate of the monosomic condition.

Materials and Methods

Data on chromosome numbers were obtained from five series of studies carried out through 1955 to 1957 to determine the frequency of transmission of

TABLE 1. Frequency of monosomics and nullisomics in progenies of selfed monosomics and from crosses of monosomic females and disomic males

Chromosome number	Frequency of selfed progeny from monosomics						Frequency from monosomic female and disomic male			
	1955		1956		1957		Pollen source 1		Pollen source 2	
	Plants tested	Freq. of mono. and nulli.	Plants tested	Freq. of mono. and nulli.	Plants tested	Freq. of mono. and nulli.	Plants tested	Freq. of mono.	Plants tested	Freq. of mono.
	no.	%	no.	%	no.	%	no.	%	no.	%
1	9	66.7	6	66.7	10	60.0	13	84.6	10	70.0
2	9	88.9	8	62.5	10	80.0	14	57.1	14	57.1
3	9	88.9	5	100.0	8	62.5	12	83.3	12	83.3
4	8	50.0	5	80.0	8	62.5	12	66.7	6	33.3
5	9	55.6	8	50.0	9	88.9	12	58.4	11	63.6
6	9	66.7	8	87.5	9	100.0	13	53.8	11	54.5
7	10	60.0	--	---*	--	---*	12	75.0	--	---*
8	9	77.8	8	62.5	10	70.0	12	66.7	11	63.6
9	9	88.9	8	62.5	10	80.0	13	46.2	10	80.0
10	9	66.7	11	63.6	9	33.3	15	40.0	11	63.6
11	9	66.7	5	80.0	10	60.0	13	46.2	7	58.4
12	8	62.5	10	70.0	10	70.0	14	92.9	11	84.6
13	7	71.4	6	83.3	10	50.0	14	57.1	10	76.7
14	10	80.0	--	---*	--	---*	14	78.6	--	---*
15	4	75.0	8	75.0	8	87.5	15	60.0	6	50.0
16	9	77.8	8	75.0	10	80.0	14	71.4	13	86.7
17	10	80.0	5	100.0	10	60.0	14	85.7	10	76.7
18	10	50.0	10	80.0	10	50.0	12	50.0	7	70.0
19	10	80.0	5	80.0	10	80.0	15	46.7	15	100.0
20	8	75.0	10	30.0	10	60.0	14	35.7	3	42.9
21	10	80.0	8	62.5	10	90.0	15	40.0	7	70.0

*No data available because nullisomic plants were used.

the monosomic condition with each of 21 wheat chromosomes. Three of these series were progenies of selfed Chinese monosomics originally established by Sears[13]. The other two were from F_1 plants of Chinese monosomics crossed with two different hexaploid pollen parents.

For the cytological determination of chromosome number, seedling plants about 20 days old were transplanted to three-inch pots, and root tips one-half inch long were collected from them one week after transplanting. Cua's pretreatment method as cited by Nishiyama[9] was used, i.e., root tips were collected in a small glass tube containing water and kept for 24 hours at a temperature of 0° C. The root tips were fixed with Farmer's solution and the Feulgen smear method was used for the chromosome stain.

The number of plants tested for each chromosome in different seasons was not the same, although the same number of seeds were planted, i.e., 10 seeds from selfing and 15 from each of the crosses. However, those numbers were fairly uniform throughout all the chromosomes studied as well as throughout the different seasons. From this viewpoint, the transmission rate of the monosomic condition was analyzed by using analysis of variance. The frequency of monosomics was transformed to angle prior to the analysis. Data for chromosomes 7 and 14 were excluded from the analysis, because those strains were generally maintained through nullisomics.

Experimental Results

Data on the occurrence of monosomics, including nullisomics, in the progenies from selfing monosomics and the frequency of monosomic plants in the progenies obtained by crossing Chinese monosomics with disomic pollen parents are given in Table I, and the results of analysis of variance on the transmission of monosomic conditions are given in Table II.

TABLE II. Analysis of variance on the transmission rates of monosomic conditions

Source of variations	df	SS	MS	
Among Series	4	832.82	208.21	NS
Selfing vs. crossing	1	544.57	544.57	*
Within selfing	2	42.38	21.19	NS
Within crossing	1	245.87	245.87	NS
Among chromosomes	18	3,743.67	207.98	*
Remainder	72	8,061.65	111.97	

NS = non-significant at the 5 percent level.
* = a significant difference at the 5 percent level.

The results of this analysis indicate that there were significant differences in the transmission rate of the monosomic condition between modes of fertilization as well as among chromosomes. A possible reason for the significant difference of transmission rates between two modes of fertilization, i.e., selfing and crossing, might be the fact that male gametes contribute, to some extent, to the transmission of the monosomic condition in selfing, but there was no such contribution by cross-fertilization with disomic pollen parents.

Significant differences among chromosomes might be due to the fact that chromosome-deficient gametes involving certain chromosomes were more viable in the process of reproduction than those of other chromosomes. Chromosomes were ranked, based on the transmission rates of the monosomic condition in average angles, and were calculated in the analysis of variance. Those average angles were adjusted for a single mode of reproduction, i.e., for selfing, for the purpose of this experiment. As the average difference of

TABLE III. Ranking of chromosomes, based on the average transmission rate of the monosomic condition in angle, and the average frequency of monosomics corrected to selfing

Chromosome	Transmission rate in angle*	Transmission rate in percent
	°	%
3	68.91	89.2
17	66.63	86.7
19	64.69	84.3
14	—	83.3**
16	62.32	81.2
6	61.76	80.4
12	61.45	80.0
9	58.50	75.7
15	57.01	73.4
Over-all mean	56.90	73.2
2	56.88	73.2
1	56.79	73.1
21	56.65	72.9
7	—	71.7**
13	55.75	71.5
8	55.71	71.4
5	53.30	67.5
11	52.33	65.9
18	51.05	63.8
4	50.14	62.2
10	47.00	56.9
20	44.32	52.2

* 5 percent L.S.D. = 13.34.
** values estimated from incomplete set of data.

transmission rates between selfed and crossed materials was 4.89 in angle, the average angle of a chromosome corrected for selfing was given in the following formula:

$$\begin{pmatrix}\text{Average angle}\\ \text{corrected for}\\ \text{selfing}\end{pmatrix} = \begin{pmatrix}\text{Average angle}\\ \text{in combined}\\ \text{data}\end{pmatrix} + 4.89 \times \frac{2}{5}$$

From this formula, the average transmission rate in selfed progenies was calculated for each chromosome and retransformed to percentage. These values are given in Table III.

As the 95 percent fiducial interval was ±9.4 for chromosome means (second column, Table III), it seemed reasonable to conclude that the transmission rates of the monosomic condition were probably higher in monosomics 3 and 17 but lower in monosomics 20 and 10 than in the average group.

Discussion

A crude classification of factors which affect the transmission of the monosomic condition can be given as follows:

(1) Exclusion of univalents from daughter nuclei in meiosis.
(2) Selection of chromosome-deficient gametes during maturation of gametophytes.
(3) Competition between normal and chromosome-deficient gametes during fertilization, and
(4) Inviability of monosomic embryos.

The frequency of tetrads with micronuclei gives information concerning the first classification. Observations of the chromosome number of mature pollen, counted at pollen mitosis, give information on the second, including the effects of the first one. Although there is no way to separate the third and fourth factors, an examination of the monosomic frequency in the progeny gives information on the third and fourth, in addition to the first two.

Using the monosomic series of Chinese Spring, Morrison and Unrau[7] observed the frequency of micronuclei in pollen tetrads, and Morrison[6] determined the chromosome number of mature pollen. The frequency of monosomics in the progeny was reported here for the same material.

So far as reliability of these results was concerned, Morrison and Unrau's data on the frequency of micronuclei were the best. Data on the monosomic frequency presented here appeared more reliable than Morrison's work, although the latter were more reliable than the former for certain of the chromosomes.

If there were little differential selection on gametes during their maturation, the frequencies of tetrads with micronuclei and chromosome-deficient gametes should be closely correlated. The frequency of chromosome-deficient gametes and the actual monosomic frequency might be clearly correlated if there were no differential competition between the various chromosome-deficient gametes and the normal ones in the process of fertilization.

Using data from Morrison[6] and Morrison and Unrau[7] and data reported on in this paper, correlations among the frequencies of pollen tetrads with micronuclei (X), the frequencies

TABLE IV. Deviation of the monosomic frequency from the range expected from the frequency of univalent exclusion

Chromosome	Frequency of monosomics	Expected frequency Upper limit : Lower limit		Deviation of mono.-freq. from nearer limit
	%	%	%	%
1	73.1	69.5	62.9	+ 3.6
2	73.2	82.6	74.9	− 1.7
3	89.2	73.9	67.2	+15.3
4	62.2	82.4	75.2	−13.0
5	67.5	77.8	71.0	− 3.5
6	80.4	74.8	66.7	+ 5.6
7	71.7	73.3	66.8	within range
8	71.4	67.2	62.3	+ 4.2
9	75.7	73.8	67.6	+ 1.9
10	56.9	67.0	62.1	− 5.2
11	65.9	73.7	67.3	− 1.4
12	80.0	70.2	64.4	+ 9.8
13	71.5	81.4	73.7	− 2.2
14	83.3	---	---	---
15	73.4	70.3	64.1	+ 3.1
16	81.2	78.7	70.7	+ 2.5
17	86.7	75.0	67.1	+11.7
18	63.8	77.6	70.8	− 7.0
19	84.3	80.1	71.2	+ 4.2
20	52.2	81.6	74.6	−22.4
21	72.9	72.4	65.9	+ 0.5

of chromosome-deficient gametes (Y), and the frequencies of monosomics (Z) were studied.

The correlation coefficient between X and Y was not significant with a value $r_{xy} = 0.133$ for df = 18, indicating that differential selection on the various chromosome-deficient gametes was more important than the exclusion of the univalents in determination of the frequency of the deficient gametes.

The correlation coefficient between Y and Z was also non-significant with a value of $r_{yz} = 0.149$ for df = 18. It was suggested that competition between gametes with different chromosome numbers during fertilization and differential viability of chromosome-deficient zygotes were more essential in determining the actual frequency of monosomics.

The correlation coefficient between X and Z was not significant, as expected from the previous results, with a value of $r_{xz} = -0.093$ for df = 18. It was possible to test the relation of X to Z from another standpoint. From Morrison and Unrau's data, one could calculate the possible upper and lower limits of the frequency of chromosome-deficient gametes, which gave, in turn, the expected limits of the monosomic frequency, when there was no differential selection.

The upper limit of the monosomic frequency expected could be obtained by assuming that all micronuclei resulted from exclusion of univalents which had not yet split. The lower limit was calculated from the assumption that all micronuclei were formed from a chromosome excluded after splitting. Actually, micronuclei were formed in both ways. The limits of monosomic frequency expected, and the deviation of the actual frequency from the nearer limit are given in Table IV.

The monosomic frequency, of all the chromosomes except chromosome 7, did not fall within the range of the expectation. The actual frequency of monosomics was either too high or too low to be explained by exclusion of the univalents. The frequencies of monosomics 3 and 17 were much higher than the expected upper limits and those of monosomics 20 and 4 were much lower than the expected lower limits. These facts suggested that deficient gametes or zygotes for chromosomes 3 and 17 were much more favorable and those of chromosomes 20 and 4 were more unfavorable than the average chromosome-deficient gametes in reproduction.

Conclusions and Summary

Experimental results on the transmission of the monosomic condition in *Triticum aestivum* var. Chinese Spring are reported.

The transmission rate of monosomics in progenies of monosomic plants was investigated cytologically in five series. The frequencies of monosomics in the selfed progenies were estimated for all 21 monosomics; these ranged from 89 percent for monosomic 3 to 52 percent for monosomic 20.

The analysis of variance of the data indicated that the transmission rate of the monosomic condition was significantly different among 19 monosomics. Monosomics 3 and 17 transmitted the monosomic condition more frequently, and monosomics 20 and 10 transmitted it less frequently than the average group of monosomics.

The importance of selection and competition among the various chromosome-deficients, during gametogenesis and fertilization, on the transmission of the monosomic condition was discussed from the standpoint that no significant correlations were found among the frequency of tetrads with micronuclei, the frequency of chromosome-deficient gametes, and the actual monosomic frequency. It was suggested that deficient gametes for chromosomes 3 and 17 were much more favored, and those of chromosomes 20 and 4 were at more of a disadvantage than the average group of chromosome-deficient gametes.

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GENETICS OF TESTA COLOR IN *CANAVALIA ENSIFORMIS*

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Canavalia ensiformis (L.) DC has been grown at this institution for many years as a cover crop and green manure. Our present stock was derived originally by the mingling of white- and light-brown-seeded strains. This genetic study began when segregation from natural hybrids had already yielded three new phenotypes in which brown testa pigment was restricted.

Rapid abscission of emasculated flowers greatly hindered crossing, but sufficient hybrids were eventually obtained. Selfing was spontaneous in bagged inflorescences, but natural crossing was evidently frequent when bags were omitted. Two crosses were found to involve one heterozygous parent, and the selfed progeny of one identifiable natural hybrid is included in the results shown in Table I.

Uniform brown testa pigment is clearly due to the complementary action of two dominant genes, designated *S* and *E*, for which white-seeded plants are recessive. Intermediate phenotypes, as illustrated in Figure 5, result

(*Continued on page 76*)

TESTA COLOR PATTERNS IN *CANAVALIA ENSIFORMIS*
Figure 5
The phenotypes are: *A*—brown (*S-E*); *B*—spotted (*S-ee*); *C*—ellipse (*ssEE*); *D*—ragged (*ssEe*); *E*—white (*ssee*).

TABLE I. Segregations for testa pigment

Cross	Genotypes	F_1	F_2 Progeny: Brown *S-E-*	Spotted *S-ee*	Ellipse *ssEE*	Ragged *ssEe*	White *ssee*	Ratio	$\chi^2[n]$	P
White × (Spotted)*	*ssee* × *Ssee*	6-*ssee*								
		6-*Ssee*		116			49	3:1	1.94[1]	0.2-0.1
Spotted × White	*SSee* × *ssee*	*Ssee*								
Ellipse × (Brown)*	*ssEE* × *SSEe*	*SsEE*	8		4			3:1	.....	.....
White × Ellipse	*ssee* × *ssEE*	*ssEe*								
Ellipse × White	*ssEE* × *ssee*	*ssEe*			88	174	75	1:2:1	1.36[2]	0.5-0.7
Natural hybrid	.....	*ssEe*								
Brown × Spotted	*SSEE* × *SSee*	*SSEe*	8	4				3:1	.....	.....
White × Brown	*ssee* × *SSEE*	*SsEe*								
Ellipse × (Brown)*	*ssEE* × *SSEe*	*SsEe*	143	43	17	32	9	9:3:1:2:1	3.24[4]	0.5-0.7
Ellipse × Spotted†	*ssEE* × *SSee*	*SsEe*								

*Bracketed parents were evidently heterozygous at one locus. In the first family, F_2's were raised from spotted segregates only.
†This was a later cross from which no F_2 was raised.

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TURKEY-CHICKEN HYBRIDS

M. W. Olsen*

ATTEMPTS have been made by a number of investigators to cross the domestic fowl and the turkey. Gray[2] lists 12 such studies and notes that in all controlled experiments there has not been a single reported case of a hybrid having hatched. Ogorodniĭ[5] reported that Spasskoy succeeded in obtaining a live hybrid, but no details or references were given. Sokolovaskaya[9], a co-worker of Ogorodniĭ, makes no reference to such a hybrid. Only infertile turkey eggs were obtained by Sokolovaskaya[9] after turkey hens were inseminated with semen of chickens or following natural matings.

Published reports of earlier workers would indicate that only a limited number of fertile eggs were obtained, and relatively few advanced embryos (15 days or older), following insemination of turkey hens with the semen of chickens. The studies of Warren and Scott[10], Quinn *et al.*[8], and Asmundson and Lorenz[1] are among the best documented reports.

Quinn *et al.*[8] incubated 278 turkey eggs, of which 56 or 20.1 percent were classified as fertile; one advanced embryo was encountered. Asmundson and Lorenz[1] incubated 119 turkey eggs of which 23 or 19.3 percent were classified as fertile, two advanced hybrid embryos having been obtained. The number of turkey eggs incubated by Warren and Scott[10] to produce one advanced embryo was not stated.

Materials and Methods

Two strains of Beltsville Small White turkey hens were used. One strain represented birds which had been selected for five generations for a high incidence of parthenogenesis[6], the other birds coming from stock in which no selection had been practiced. Hens of the two strains were further identified as virgin or previously mated, an interval of not less than eight months intervening between last mating and use of the hens in this study.

Two breeds of chicken males were used—Dark Cornish from a line which had been selected for several generations for a high incidence of parthenogenesis and Rhode Island Red from stock which had not been selected for this trait.

Turkey females were inseminated at intervals of from two to 10 days with 0.1 ml. of chicken sperm. Five groups (or matings) are identified as follows:

Group A—Selected virgin hens × Dark Cornish males

Group B—Selected previously mated hens × Dark Cornish males

Group C—Selected virgin hens × Rhode Island Red males

Group D—Unselected virgin hens × Dark Cornish males

Group E—Unselected previously mated hens of Group D × Rhode Island Red males

The plumage color of both the Dark Cornish and the Rhode Island Red is dominant to the recessive white of the Beltsville Small White turkey. Thus, a genetic color marker was present making it possible to establish the parentage of embryos 15 days or older.

Pedigreed eggs were set on the day they were laid and incubated at a temperature of 99.5° F. and a relative humidity of 57 percent. All eggs were candled on the ninth or tenth day of incubation, at which time any eggs containing live embryos were returned to the incubator. Eggs which failed to show embryonic development or those in which the embryos had died were removed, broken-out and a macroscopic examination made of the germinal disc for evidence of development. Eggs in which no discernible enlargement of the germinal disc was noted were considered to be infertile. The remaining eggs were

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TABLE I. Incubation record of eggs laid by virgin and previously mated Beltsville Small White turkey hens following insemination with semen from Dark Cornish or Rhode Island Red chicken males

Group	*Selected turkey hens*						*Unselected turkey hens*			
	A		B		C		D		E	
Breed of chicken males	Dark Cornish		Dark Cornish		Rhode Island Red		Dark Cornish		Rhode Island Red*	
History of female	Virgin		Previously mated		Virgin		Virgin			
Total number hens	71		77		4		24		24	
Total number eggs	609		997		42		296		188	
	No.	%	No.	%	No.	%	No.	%	No.	%
Eggs showing development	368	60.4	499	50.1	25	59.5	177	60.5	42	22.3
Membranes without blood	159	26.1	297	29.8	14	33.3	29	9.8	27	14.4
Membranes with blood	87	14.3	101	10.1	5	11.9	82	27.7	8	4.3
Embryos	122	20.0	101	10.1	6	14.3	66	23.0	7	3.7
Ages attained by embryos										
2-7 days	66	54.1	51	50.5	3	50.0	30	45.4	4	57.2
8-14 days	7	5.7	14	13.9	3	50.0	3	4.5	1	14.3
15-21 days	11	9.0	9	8.9	0		10	15.2	0	
22-28 days	27	22.2	17	16.8	0		21	31.8	2	28.5
Hatched	11	9.0	10	9.9	0		2	3.0	0	
Total number embryos	122	100.0	101	100.0	6	100.0	66	100.0	7	100.0

*These birds are the same as those shown in Group D. They were previously inseminated with Dark Cornish semen, but never were mated to turkeys.

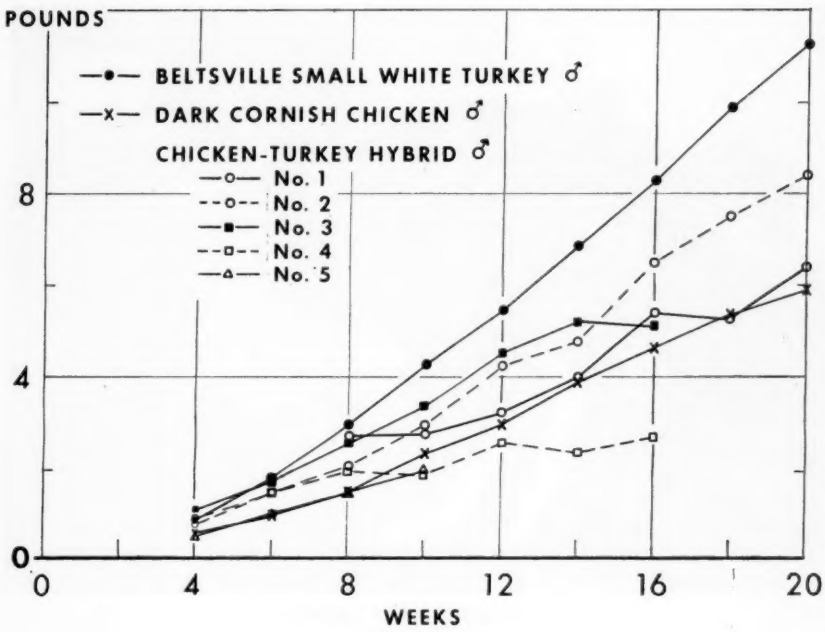
classified into three categories on the basis of degree of development: (1) eggs in which only embryonic membranes were identified, (2) those in which blood vessels had formed in addition to membranes and (3) those containing well-formed embryos, which were further classified as to age at time of death in relation to the size of a normal turkey embryo. The down color and sex, based on examination of gonads, of all embryos 15 days or older as well as those of hatched poults were recorded. Although all hybrids encountered had to be helped from the shell, they will be referred to subsequently as having hatched.

Results

Results are summarized in Table I. In both virgin and previously mated hens, chicken semen was moderately effective in initiating development capable, in some cases, of proceeding to embryo formation. Approximately 60 percent of the eggs from virgins (Group A) and 50 percent of those from previously mated hens (Group B) showed some degree of development. Most of this represented an unorganized type of growth in which only membranes or, in some instances, membranes and blood developed.

Embryos, varying in ages from three to 25 days, were found in 20 percent of the eggs from selected virgins and in 10.1 percent of the eggs from previously mated selected hens crossed with Dark Cornish males. Among the 122 embryos encountered in eggs from virgin hens (Group A), 49 or 40.1 percent attained an age at which down color served to establish that they were hybrids. Eleven hybrids survived until time of hatching (22 to 25 days) and were helped from the shell.

Of the 101 embryos from older hens (Group B) 36, including 10 which hatched, or approximately 35 percent attained an age at which down color established that they were sired by Dark Cornish males. Thus, a total of 21 turkey-chicken hybrids hatched from eggs of hens of Groups A and B. Nearly all were weak, and almost invariably



GROWTH CURVES

Figure 6

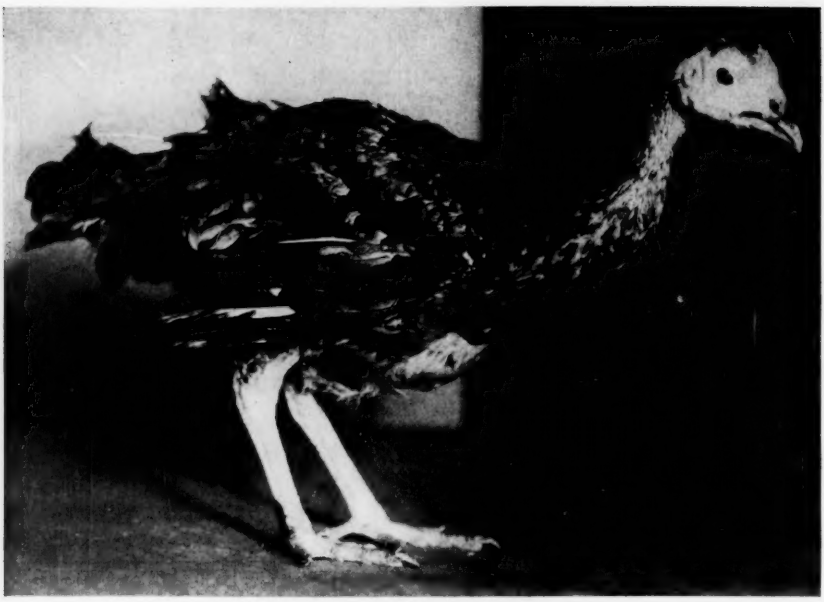
Body weights of five individual turkey-chicken hybrids and average body weights of Dark Cornish chicken and Beltsville Small White males. Weights were determined at intervals of two weeks when birds were one to five months of age.

exhibited defects. Crooked beaks, microphthalmia, crooked legs and toes were among the commonly occurring defects. Few hybrids survived longer than 24 hours after hatching.

Unselected turkey hens, when mated to selected Dark Cornish males (Group D), were also capable of producing eggs of which a relatively high percentage (23 percent) contained embryos. Two hybrids (1.1 percent) hatched. However, when the same hens were subsequently inseminated with semen from non-selected Rhode Island Red males (Group E) only 22 percent of the eggs showed development and only 3.7 percent were found to contain embryos, all of which died in the shell. The percentage of embryos is thus six times greater in eggs of the same hens with semen from Dark Cornish rather than Rhode Island Red males.

Semen from the Rhode Island Red males was used to inseminate the four selected hens of Group C. Nearly 60 percent of all eggs tested showed some degree of development and 14.3 percent contained embryos. This is nearly three times the percentage of development and nearly four times the percentage of embryos encountered in eggs from unselected females (Group E) mated to the same males. These data indicate that the type of the female, as well as of the male, exerts an influence on the degree of viability of the embryo.

The oldest hybrid died at 21 weeks of age. Four hybrids are still alive, three originating from eggs laid by selected hens of Groups A and B and one from eggs of non-selected hens of Group D. Growth curves of these hybrids as well as for representative groups of chicken and turkey males are given in Figure 6.



TURKEY-CHICKEN HYBRID

Figure 7

Turkey-chicken hybrid at 20 weeks of age weighing 5.5 pounds.

Marked differences exist between individual hybrids in rate of growth, these becoming more pronounced with age. Three of five hybrids have body weights intermediate to those of chicken and turkey males of similar age. Two hybrids have failed to attain the average weight of chicken males. Three of the four living hybrids have visible defects which include crossed beaks, notched upper beaks, and curled tongues. The fourth hybrid, shown in Figure 7, is free of these abnormalities. Each hybrid was hand raised, forced feeding still being required in the case of three birds.

In appearance, the turkey-chicken hybrids, including both the hatched and unhatched, resemble the chick more than the turkey poult. Each has shown the color markings, the short heavy legs and the fully feathered neck of the chicken males used, but the white skin of the turkey. All hybrids thus far encountered have had flattened, cushion-type combs minus spikes. Furthermore, appendages such as snoods, wattles, and earlobes were missing. The hybrid shown in Figure 7 is a typical example. All advanced hybrid embryos including those which have hatched (where sex was determined) were males. The testes were approximately twice the size of those of a day-old chick and three or four times the size of those of a newly hatched poult.

Discussion

It is evident that, under certain conditions, spermatozoa from Dark Cornish and Rhode Island Red males are capable of fertilizing turkey eggs. The resulting zygotes on rare occasions can develop to hatching or even, in some instances, survive for some time thereafter. This is true in spite of marked differences in chromosome number between the two families, six pairs of macro-chromosomes for the chicken (Newcomer[4]) and nine pairs for the turkey (Kosin[3], Poole[7]), and in differing lengths of the incubation period.

As was noted earlier, all advanced hybrid embryos, including those which have hatched but died, have been males. The two hybrid embryos encountered by Asmundson and Lorenz were likewise males (personal com-

munication). The sex of the one hybrid embryo found by Quinn *et al.*[8] and the one encountered by Warren and Scott[10] was not reported.

In this study, 5.6 percent of the total eggs set contained advanced embryos, an incidence more than seven times greater than that obtained by the two groups of earlier investigators[1, 8]. The question naturally arises as to why, in this particular study, viable hybrids and many advanced embryos have been produced while in other investigations only a few advanced embryos were encountered, none of which hatched. It would seem that characteristics of the breeding stock, both males and females, exert a controlling influence in the production of turkey-chicken hybrids. Certain distinguishing features of the present stock and those used by the three groups of earlier investigators[1, 8, 10] may be noted. The Dark Cornish males as well as the Beltsville Small White turkeys came from lines of birds which had been selected for several generations for a high incidence of parthenogenesis[6]. The selected hens, prior to their use in these studies, laid unfertilized eggs, 6.9 percent of which upon incubation produced well-developed parthenogenetic embryos. The Rhode Island Red, White Wyandotte and White Leghorn chicken males used in earlier investigations were not selected for this trait. This was likewise true of the Standard-Bred Bronze, Broad Breasted Bronze and the White Holland turkey females used by earlier workers.

Summary

Beltsville Small White turkeys were inseminated with semen of Dark Cornish and unselected Rhode Island Red males. Selection of parent stock for an increased incidence of parthenogenesis appeared to enhance embryonic viability. Approximately 60 percent of eggs from selected females × selected males showed embryonic development. Approximately the same percentage was encountered in eggs from reciprocal matings of unselected females × selected males. Only 22 percent of the eggs from unselected hens mated to unselected cocks showed embryonic development.

A total of 302 embryos (14.2 percent) were encountered among 2,132 eggs incubated. One-hundred-twenty of these embryos attained an age at which down color established hybridization. All hybrids, including advanced embryos, thus far examined for sex have been males with Dark Cornish or Rhode Island Red coloration; all had white skin, lacked both snoods and wattles and had fully feathered necks. Four of 23 hybrids hatched are alive, ranging in age from nine to 19 weeks and in weight from 1.75 to 8.0 pounds.

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GENETIC BLOCKS IN THE SYNTHESIS OF COUMARIN

In *Melilotus alba*

H. J. Gorz and F. A. Haskins*

COUMARIN synthesis in sweetclover (*Melilotus alba*) appears to be primarily controlled by two independent pairs of genes designated as *Cu/cu* and *B/b*[1, 3]. When homozygous, the *cu* gene produces a marked reduction in the level of total coumarin while the *b* gene prevents the formation or accumulation of appreciable quantities of free coumarin in the plant. Plants of the *Cu B* phenotype contain both free and bound coumarin, plants of the *Cu b* phenotype contain bound coumarin but are very low in free coumarin, and plants of the *cu B* and *cu b* phenotypes are low in both forms of coumarin. It is reasonable to suppose that the two gene pairs affect, either directly or indirectly, certain steps in the biosynthesis of the coumarin molecule. In the terminology of the chemical geneticist, it might be said that the *cu* and *b* genes, when homozygous, block specific reactions concerned with coumarin synthesis. Such biosynthetic blocks are well known, particularly in *Neurospora crassa* and other microorganisms[5]. It is the purpose of this paper to present the results of experiments in which improved assay methods have been used to measure the influence of the two genes, *cu* and *b*, upon the level and form, respectively, of coumarin in sweetclover leaf tissue.

Materials and Methods

Twenty-five sweetclover plants of each of the four homozygous genotypes, *CuCuBB*, *CuCubb*, *cucuBB*, and *cucubb*, were used in this study. The plants of known genotype were chosen from several F_8 lines that had been derived from a single, doubly heterozygous F_6 plant. In the original cross from which this F_6 plant was derived, a *cucuBB* plant was used as the female parent and a *CuCubb* plant as the male parent. (Acknowledgment is made to Dr. W. K. Smith at the University of Wisconsin for the parental material.) Ten alfalfa plants also were assayed to provide an indication of the magnitude of fluorescence not ascribable to coumarin. Assays for free and bound coumarin were made with the use of the Beckman model DU spectrophotometer equipped with the fluorescence attachment. Young leaf tissue was used, and the procedure described by Haskins and Gorz[2] was followed.

Results and Discussion

Average levels of free, bound, and total coumarin, with standard errors, for each of the four homozygous genotypes of sweetclover and for alfalfa are shown in Table I. In Table II, levels of free and bound coumarin are expressed as percentages of the total coumarin content.

TABLE I. Average coumarin content of sweetclover leaves representing plants of four genotypes and of alfalfa leaves

	Coumarin equivalence (dry weight basis)		
	Free	Bound	Total
Genotype	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
	%	%	%
cucubb	0.02 — .002	0.20 — .022	0.22 — .022
cucuBB	0.08 — .013	0.16 — .030	0.24 — .037
CuCubb	0.02 — .001	5.13 — .268	5.15 — .268
CuCuBB	1.09 — .102	3.05 — .315	4.14 — .317
Alfalfa	0.01 — .001	0.01 — .001	0.02 — .001

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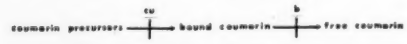
It is apparent from the data that in plants which are homozygous with respect to *cu*, the synthesis of coumarin is not completely blocked. Although the average level of total coumarin in *cucu* plants is appreciable and easily detectable, amounting to approximately 0.2 percent of the dry weight, the level is only about 1/20 of that found in *CuCu* plants. The degree of fluorescence noted in assays of *cucu* plants, however, is 11.5 times that observed for alfalfa, and it must be concluded that the *cu*-effected block in coumarin synthesis is partial rather than complete.

The *b* gene, on the other hand, appears to be highly effective in blocking the formation of free coumarin. Thus, in plants of the *CuCubb* genotype, less than one percent of the total coumarin content is in the form of free coumarin. In *cucubb* plants the proportion of free coumarin appears to be somewhat larger, amounting to about nine percent. However, for both of these genotypes the free coumarin levels listed in Table I are almost as low as the value recorded for alfalfa. It is probable, therefore, that little if any of the "free coumarin" fluorescence noted in assays involving these genotypes is actually due to coumarin. This very small amount of "free coumarin" accounts for a greater percentage of the total coumarin content in plants of the *cucubb* genotype than in *CuCubb* plants because of the low total coumarin content of the former plants. In view of these considerations, it may be assumed that the values of 0.4 percent free coumarin and 99.6 percent bound coumarin listed in Table II for the *CuCubb* genotype probably represent the upper limit for the free form and the lower limit for the bound form, respectively, in plants which are homozygous for *b*. Thus, a virtually complete blocking action is indicated for the *b* gene.

Information thus far available indicates that bound coumarin, free coumarin, and the *cu* and *b* genes are related as follows:



As indicated in this proposed pathway, bound coumarin appears to serve as a precursor of free coumarin in the plant, the *cu* gene partially blocks the conversion of unspecified precursors to bound coumarin, and the *b* gene prevents the conversion of bound coumarin to the free form. Further work on this biosynthetic pathway is planned.

The results reported in this paper have an important bearing on sweetclover breeding. One of the primary objectives in sweetclover breeding programs is the development of strains that are free of coumarin, since this chemical imparts a bitter taste to the forage and also gives rise to the toxic material, dicoumarol, which occurs in spoiled sweetclover hay and silage. Dicoumarol is the causative agent associated with the so-called "sweetclover disease" in livestock[4]. Breeding for low coumarin level requires the analysis of many thousands of plants for coumarin content. Of necessity this large-scale analysis must be done qualitatively. Qualitative fluorometric procedures[2] now available for use by the plant breeder permit the detection of very low levels of coumarin. The plant breeder should be aware that the *cu*-effected block in coumarin synthesis is partial in nature, and that some coumarin should, therefore, be expected in plants having the *cucu* genotype. An application of this knowledge may save much valuable germplasm that might otherwise be discarded as unsuitable.

It is also important to know the level of coumarin in plants of each of the various genotypes when utilizing the coumarin-conditioning alleles as genetic markers, or in studies of the physiology of coumarin synthesis in sweetclover.

Summary

The influence of the two genes, *cu* and *b*, upon the level and form of coumarin in sweetclover leaf tissue was determined by assaying 25 sweetclover plants of each of the four homozygous genotypes, *CuCuBB*, *CuCubb*, *cucuBB*, and *cucubb*. An assay of alfalfa leaves provided an indication of the magnitude of fluorescence not ascribable to coumarin. Approximately 0.2 percent total coumarin (dry weight basis) was found in plants homozygous for *cu*, which is 11.5 times the amount found in alfalfa, but only about 1/20 of the level found in *CuCu* plants. Thus, the *cu*-effected block in coumarin synthesis is partial rather than complete. However, the action of the *b* gene in blocking the formation of free coumarin is virtually complete, as shown by the extremely low levels of free coumarin in *bb* plants. The probable relationship of bound coumarin, free coumarin and the *cu* and *b* genes is shown. Important implications in sweetclover breeding, and in other studies are indicated.

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TABLE II. Levels of free and bound coumarin in four genotypes of sweetclover, expressed as percentages of total coumarin content

Genotype	Total coumarin	Percentage of total content occurring as	
		Free	Bound
	% of dry wt.		
cucubb	0.22	9.1	90.9
cucuBB	0.24	33.3	66.7
CuCubb	5.15	0.4	99.6
CuCuBB	4.14	26.3	73.7

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Genetics of Testa Color in *Canavalia ensiformis*

(*Continued from page 68*)

from the separate action of those genes as follows:

S - ee = "spotted": seeds spotted with brown in a characteristic lateral and dorsal pattern;

ss EE = "ellipse": seeds brown except for a white ellipse around the hilum;

ss Ee = "ragged": similar to the preceding but the ellipse is larger and less definite and the brown area is flecked with white.

Pigment intensity was variable, but no genetic basis for this was discerned.

From a small sample of plants it was apparent that the gene pair *S,s* also controlled a difference in flower color. The flowers of *S-* plants were uniformly red-purple; those of *ss* plants were whitish towards the base.

Genes for restriction of testa pigment in legumes have been reported as members of a multiple allele series either at the locus of a basic pigment gene as in *Phaseolus*[1], or at an independent locus, as in *Glycine max*[2]. However, in *Vigna sinensis*, Smith[3] has shown that self-color is a complementary effect of the restrictive Watson (*W*) and Holstein (*H*) genes, a case exactly analogous with the present one.

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"HYDRODORSAL" AND OTHER FETAL MALFORMATIONS IN SWINE

E. S. E. Hafez*

SEVERAL hereditary factors cause such abnormal development in the embryo that the fetus fails to develop properly, atrophies, or dies. Among these cases the degree of abnormality varies greatly, although the gene which produces it is the same. Probably the effect depends upon the stage of development of the embryo at which the defective gene comes into operation; thus, if the fetus is slow in developing, the abnormality is marked, whereas if the factor takes effect at an advanced stage of development the abnormality is slight.

Swine reproduce at a relatively high rate and under normal physiological conditions, they do not abort dead fetuses. These facts account for the high incidence of lethals and abnormalities in this species. Several lethals and sub-lethals have been described for swine fetuses. These abnormalities can be physiological or anatomical. Physiological abnormalities actually are due to inherited abnormal structure of some gland, organ or tissue such as the endocrine glands, nervous system or blood.

The "Palouse" meat-type breed of pigs was initiated in 1945 by crossing Landrace boars to Chester White sows. The gene distribution of the new breed was moved more heavily toward the Landrace by backcrossing to it. The genealogy of the "Palouse" breed has been adequately described[1, 2]. The breed carries considerable length of body, and some individuals possess an extra pair of ribs. Moderate depth of body and a neatly-turned loin are characteristic of the breed. The arch of back is moderate, and the underline is straight and smooth. Weak feet, legs or defective backs sometimes occur, as they do in several of the other inbred breeds based on the Landrace crosses.

The purpose of this paper is to report the types of fetal malformations which may be related to inborn weaknesses observed in some individuals in the "Palouse" breed.

Materials and Methods

In this investigation 217 fetuses (38 days) and 120 fetuses (100 days) were available. Thirty-six sows were mated at the third pubertal estrus to five fertile boars. The pregnant sows were slaughtered 38 and 100 days postcoitum. The genital tract was removed immediately after slaughter. The ovaries were removed and the number of corpora lutea recorded. The number of implantations was recorded. The gravid uterus was cut open from the tubal end and the fetuses were removed, dissected and internal organs carefully examined. A fetus was considered normal if it had no gross abnormalities or apparent degeneration of its tissues or internal organs. The animals were derived from four lines. The lines were closed to outside blood; however, inbreeding was kept at a minimum by systematic crossing of the groups within lines, with no mating closer than between quadruple second cousins. The same system of mating was practiced in each line in order to keep the inbreeding coefficients as nearly alike as possible.

*Scientific Paper No. 1864, Washington Agricultural Experiment Station, Department of Animal Science, Pullman, Washington. Project No. 807. Grateful acknowledgments are due to the Rockefeller Foundation, New York, N. Y. This work has been aided by grants from Swift and Company, Chicago, Illinois, with added support from Carstens Packing Company, Tacoma and Spokane, Washington; Gibson Packing Company, Yakima, Washington; Pendleton Grain Growers, Pendleton, Oregon; American Dry Milk Institute, Inc., Chicago, Illinois; Hiram Walker, Peoria, Illinois; Standard Brands, Inc., New York, N. Y.; and Charles Pfizer and Co., Brooklyn, N. Y.

Results and Discussion

The losses, expressed as percentage of the total number of corpora lutea, were: ova, 16.22; embryos, 15.83; and fetuses, 8.72 (Table I). The total prenatal loss was 40.77 percent; this was due to hereditary and environmental (maternal) factors. The development of the fetus is under the control of several genes and malformation may result from a faulty maternal environment.

a. Fetal "Hydrodorsal"

Thirteen fetuses (38 days) from three sows showed dorsal subcutaneous vesicles filled with watery fluid. The dorsal area was slightly edemic but the fetuses appeared to be otherwise normal in size and conformation. The vesicle varied in size (Figure 8) and was easily punctured. The fluid, when examined microscopically, did not show any cellular components, but contained a few fat droplets. Serial cross-sections were made of the affected embryos and it appeared that the internal structure was normal and not retarded (Figure 10). The stage at which malformation begins was not determined. There was indication that the defect has a genetic background and that the maternal environment had little influence on the defect. The three sows which showed these embryonic defects were related and they could be traced to two particular boars (Nos. 117 and 225). "Hydrodorsal" vesicle appeared irrespective of the sequence in the uterine horn.

It is interesting to note that the "hydrodorsal" vesicle did not appear in the 100-day fetuses. Three of the 100-day litters were sired by boar No. 117 and two litters were sired by boar No. 225. All these 100-day fetuses apparently were normal. However, six of the fetuses weighed below the average fetal weight at 100 days. It would appear that a certain threshold during fetal development is involved in the appearance of the "hydrodorsal."

TABLE I. Incidence of fetal malformation and atrophy in swine at different stages of pregnancy

Items	Stage of pregnancy 38 days	100 days
Total number of corpora lutea	259	195
Total number of observed implantations	217	120
Total number of malformed[1] fetuses	41	17
Ova[2] loss %[5]	16.22	
Embryo[3] loss %[5]	15.83	
Fetus[4] loss %[5]	8.72	
Total loss %	40.77	

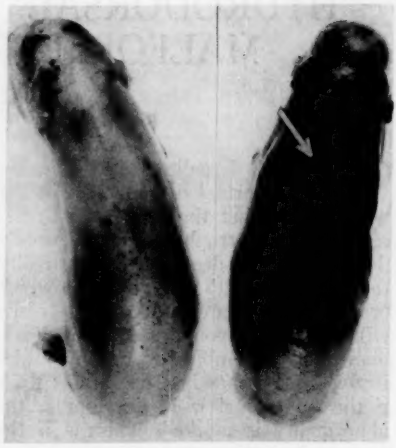
[1]This includes fetal atrophy.

[2]This includes malformed ova, unfertilized ova, uncleaved ova and very early degeneration of implanted ova.

[3]This includes detectable loss from implantation to 38 days of pregnancy.

[4]This includes the detectable loss between 38 and 100 days of pregnancy.

[5]Expressed as a percentage to total number of corpora lutea.



"HYDRODORSAL" VESICLE IN 38-DAY SWINE FETUSES

Figure 8

Dorsal view of a normal fetus (left), and a completely formed vesicle in a defective fetus (right). The arrow shows the location of the dorsal vesicle.

b. Cyclopia

Sow No. 28 was slaughtered 100 days postcoitum. The right horn had seven viable fetuses and one dead fetus in the middle of the horn. The left horn had two fetuses, a cyclops (having one eye in the middle of the forehead) near the cervical end, and one normal fetus near the tubal end. The cyclops (Figure 9) was normal in size and weighed 774 gm. The normal fetuses of the litter weighed from 468 to 1110 gm. The cyclops was viable but slightly retarded in development as indicated by the growth of body hair. It also showed brachygnathia and an elephant-like proboscis. Similar cases have been observed by Holz and Fortuin[4].

c. Muscular dystrophy

Muscular degeneration was observed in one 38-day fetus which was characterized by discoloration of the musculature, which was grayish. A similar condition has been described in pigs[5].

d. Other fetal abnormalities

Other cases of fetal abnormality also were observed: strangulation by the umbilical cord (three cases), detached umbilical cord (one case), umbilical hernia (one case) and kera-

FETAL CYCLOPIA

Figure 9

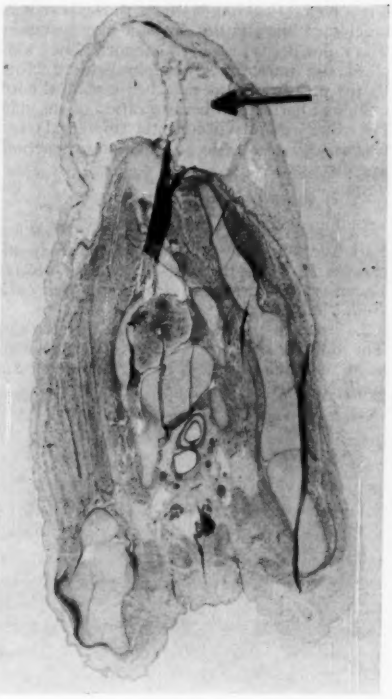
A head of normal 100-day fetus (upper). A head of 100-day cyclops showing brachygnathia and an elephant-like proboscis (lower).

tized skin (one case). In 100-day fetuses, the liver and gall bladder showed more malformation than the other internal organs which were examined. Cysts of five to 12 mm. in diameter were attached to the liver (14 fetuses) or the gall bladder (two fetuses). Not all the fetuses of the same litter were affected. Urogenital malformations also have been reported in swine fetuses[7]. Some of the fetal malformations, such as fetal blindness[6, 8], are due to genetic × nutrition interaction.

e. Fetal atrophy

Fetal atrophy was recorded at 38 days and 100 days of pregnancy. There was no indication that the degree of fetal atrophy is affected by the sequence of embryo in the uterine horn. However, in one sow the left side of the litter was completely degenerated, and reabsorption was more advanced near the tubal end than near the cervical end. As for the reabsorption rate of the different parts of the fetus, the skull seemed to be the slowest.

Causes of fetal atrophy could be classified into immediate and intermediate causes. The immediate (direct) causes may be related to defective sperm or ova, defective fertilization, segmentation, implantation, placentation or vascularization or to embryonic disorientation. These causes are mediated by genetical, hematological, nutritional, endocrinological, endometrial or pathological factors. The predisposing factors for fetal atrophy and their relative importance are as yet largely unknown. It is also suggested that stillbirth and neonatal mortality are controlled by genetic factors causing defective abnormalities in the fetus at late stages of development. It has been reported that the German Edelschwein breed produces more stilbirths than the Large White breed; related parents have more stillbirths than unrelated parents[3].



"HYDRODORSAL" VESICLE

Figure 10

A microscopic preparation of a cross-section of a 38-day fetus afflicted with "hydrodorsal" vesicle. Note the subcutaneous vesicle filled with fluid. The internal organs were embryologically normal.

Summary

Anatomical studies were carried out on 217 fetuses of known age (38 and 100 days). The losses, expressed as percentage of the total number of corpora lutea, were: ova, 16.22; embryos, 15.83; and fetuses, 8.72. Fetal "hydrodorsal" vesicle was observed in 13 fetuses. The defect was not associated with abnormal embryogenesis; a threshold during development

is involved. One 100-day fetus showed cyclopia, brachygnathia and an elephant-like proboscis. The following types of fetal abnormalities were observed: muscular dystrophy, strangulation by the umbilical cord, detached umbilical cord, umbilical hernia and keratinization of the skin. Hereditary and environmental (maternal) factors as well as genetic × nutrition interactions are discussed.

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AN INTERSPECIFIC HYBRID IN PASPALUM

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COMMON Dallisgrass, *Paspalum dilatatum* Poir., is a widely adapted pasture plant in the South. Compatibility with other species and persistence under close grazing make it useful for a wide range of conditions. Poor seed quality, susceptibility to ergot (*Claviceps paspali* Stevens & Hall) and low seed production are the main factors limiting its more widespread use[3].

The formation of multiple embryo sacs and meiotic irregularity have been suggested as the causes of poor seed set[1, 2, 9]. Common Dallisgrass has $2n = 50$ chromosomes consisting of 20 bivalents and 10 univalents at metaphase I[1]. Meiosis is extremely irregular with 10 lagging univalents at anaphase I. It was found recently[2] that cells of the nucellus form aposporus embryo sacs which frequently produce proembryos prior to anthesis. However, pollination is necessary for endosperm development. The absence of aneuploids and the uniformity of progeny were considered as evidence for obligate apomixis. This would account for the failure of conventional breeding programs to produce significant improvements in seed quality.

Paspalum malacophyllum Trin., a species introduced from South America, produces good-quality, ergot-free seed[7]. It is not suitable as a substitute for Dallisgrass in a pasture program because it will not persist under grazing. Burton[5] listed the chromosome number of *P. malacophyllum* as $2n = 40$. Studies of the meiotic behavior of this species were included in this investigation.

P. malacophyllum was used in a hybridization program with Dallisgrass in an attempt to incorporate immunity from ergot and high seed set into a single line. Differences in time of flowering, pollination, and the impossibility of emasculation under natural conditions of flowering were overcome by temperature and light control. This paper reports the results of a cytogenetic investigation of an F_1 hybrid between these two species and three of the F_2 segregates.

Materials and Methods

The method used in crossing these two species has been described[4]. Potted plants of each species were allowed to complete the third day of flowering. Florets that had bloomed were removed from inflorescences of the female parent and all plants were placed in a control room (55° to 60° F.) at 6 p.m. All florets opening on the inflorescences to be used as the female parent were emasculated by means of forceps. The emasculated plants were removed to another room at approximately 80°. The pollen plants were removed from the control room and quickly placed next to the plants were removed from the control ing racemes. Anthers dehisced within five minutes after removal from the control room. The non-flowering spikelets were removed and the pollinated inflorescences were covered with a glassine bag.

Material for cytological studies was killed and fixed in 3:1 alcohol-acetic acid. Anthers and root tips were smeared by the standard iron aceto-carmine techniques. Chromosome counts were made from anaphase I preparations and confirmed from root-tip smears. Cytological studies of the F_1, three F_2 progeny and of *P. malacophyllum* were made.

Results

A species hybrid was obtained from over 2,000 pollinations when *P. dilatatum* was used as the pistillate parent. No

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seed was obtained from approximately the same number of pollinations of the emasculated *P. malacophyllum* florets. Several morphological characteristics of the parental species and their F_1 are described (Table I).

The dominant characters from the *P. malacophyllum* parent are the shape and size of the florets, the inflorescence and the leaf-sheath markings. Dominant characters from the *P. dilatatum* parent are anther and stigma color, plant type, length of culms, and the glabrous leaf sheath.

The hybrid exhibits hybrid vigor to a great extent. Culm counts per plant averaged 61 for *P. malacophyllum*, 73 for *P. dilatatum*, and 130 for the F_1. Forage production expressed as dry matter per plant showed 1.1 pounds for *P. malacophyllum*, 1.4 for *P. dilatatum*, and 5.3 for the hybrid.

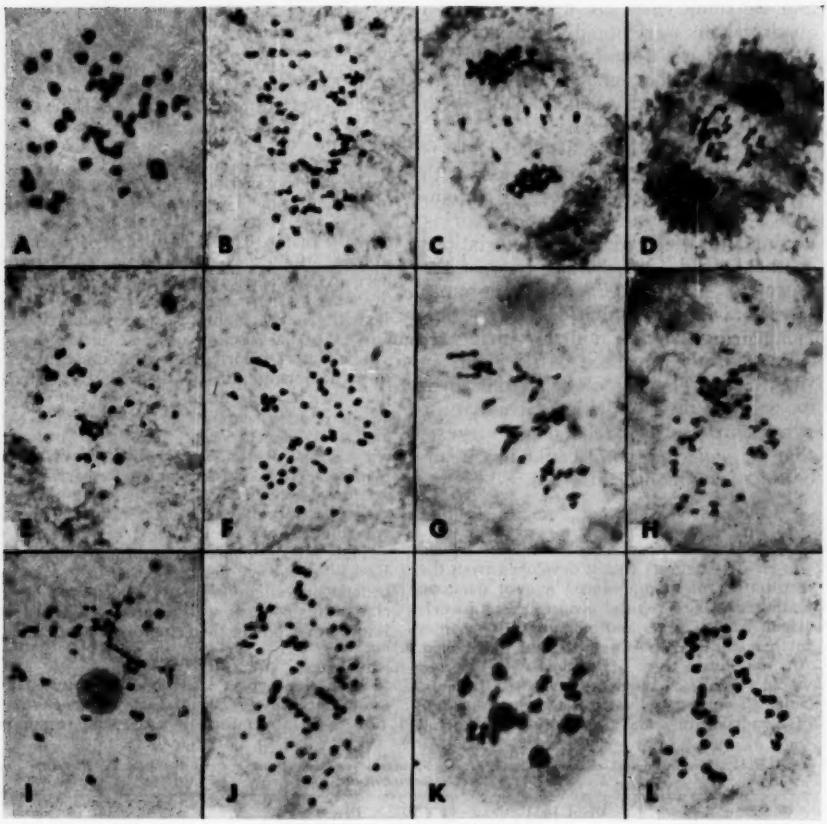
Morphological characteristics of three F_2 plants are given in Table II.

Observations for Table II were taken from three widely varying ergot-immune and highly resistant segregates obtained from the F_1. Segregate 1431 more closely resembles *P. malacophyllum*, 1432 is intermediate between *P. malacophyllum* and *P. dilatatum*, and 1433 resembles *P. dilatatum*. The F_2 progeny segregated toward *P. dilatatum* and *P. malacophyllum* type plants in the proportion of 369 to 93. Hybrid vigor is shown by the F_2 plants reported here. Culm counts per plant were 91 for 1431, 85 for 1432, and 77 for 1433.

The chromosome number of the F_1 was found to be $2n = 70$ (Figure 11*B*). In view of the chromosome numbers of the parents (common Dallisgrass, $2n = 50$, and *P. malacophyllum*, $2n = 40$), it appears certain that the F_1 was derived from fertilization of an unreduced egg of common Dallisgrass. In approximately 200 cells observed at diakinesis and metaphase I the number of elements ranged from 35 to 40, and at least five quadrivalents were present in all cases (Figure 11*A*). Approximately 35 percent of these cells contained 40 elements which were interpreted as five quadrivalents, 15 bivalents, and 20 univalents. In cells containing less than 40 elements the reduction in number was due to the presence of trivalents and occasionally additional quadrivalents. The F_1 was characterized by extreme meiotic irregularity. Anaphase I cells contained 10 to 20 laggards which were still obvious at late telophase (Figure 11*C* and *D*). It is likely that many lagging chromosomes are lost and inviable male gametes may result, which may account for the low seed set observed.

TABLE I. Morphological characteristics of *Paspalum dilatatum*, *P. malacophyllum*, and their F_1 hybrid

Character	*P. dilatatum*	*P. malacophyllum*	F_1
Culms	from a decumbent base	semi-decumbent	erect
Blade	glabrous or with a few tufts of hair in ligule areas, 4-12 mm. wide, 10-30 cm. long	sparsely pilose on both surfaces, copiously ringed with white hairs behind ligule, 8-15 mm. wide to 45 cm. long	glabrous, tufts of hair in ligule area, 20-30 mm. wide to 60 cm. long
Ligule	acute, membranous, mottled, 2 mm. tall	rounded, membranous, slightly mottled, 2 mm. tall	rounded, membranous, 6 mm. tall
Collar	glabrous, broad, continuous	glabrous, narrow, continuous, tufted at edges	glabrous, broad, continuous, tufted at edges
Sheath	compressed, slightly keeled, conspicuously pilose near base, otherwise glabrous, margins loosely overlapping	round, papillose-pilose, overlapping margins, villose	compressed, overlapping margins, conspicuously villose
Inflorescence	racemes 3-9, spreading, 6-8 cm. long, spikelets 3-4 mm. long, fringed with long white hairs	racemes 10-45, pale to purple, in nodding panicle, 3-4 cm. long, spikelets 1.5-2 mm. long, fertile lemma strongly ridged	racemes 10-60, spreading, 8-12 cm. long, spikelets 1.5-4 mm. long
Anther color	purple	red and reddish yellow	purple
Ergot reaction	completely susceptible	immune	highly resistant



MEIOTIC CHROMOSOMES
Figure 11

Meiotic figures in a common Dallisgrass × *Paspalum malacophyllum* F_1 hybrid, in an F_2 progeny and in *P. malacophyllum*. *A-D*—F_1 hybrid showing multivalent associations, bivalents and univalents at metaphase (*A*), 70 chromosomes at anaphase (*B*) and lagging chromosomes at anaphase and telophase (*C* and *D*). *E* and *F*—Metaphase and anaphase in F_2 line 1431. *G* and *H*—Metaphase and anaphase in F_2 line 1432. *I* and *J*—Diakinesis and anaphase in F_2 line 1433. *K* and *L*—*P. malacophyllum* showing six quadrivalents and eight bivalents at diakinesis (*K*) and 40 chromosomes at anaphase (*L*).

The F_2 progeny examined in this study had chromosome numbers of 52, 54, and 65, respectively (Figure 11*F*, *H* and *J*). Multivalent associations (Figure 11*E*, *G* and *I*) were also apparent in diakinesis and metaphase I cells of the F_2 plants, but there were fewer univalents than in the F_1. A variable number of laggards were observed at anaphase I in the F_2 progeny, but no attempt was made to determine the characteristic number for each plant. The aneuploid nature of the F_2 progeny indicates that the F_1 reproduces sexually.

The cytology of the male parent species provides a reasonable explanation for the presence of multivalent associations in the hybrid. Diakinesis and metaphase I cells of *P. malacophyllum* showed a high percentage of quadrivalents. In over 50 percent of the observations there were five quadrivalents and 10 bivalents,

and in some cases as many as seven quadrivalents were noted (Figure 11*K*). Anaphase I was found to be regular except for the occasional observation of one or two laggards which usually reach the poles by early telophase.

It is probable that half-quadrivalents of *P. malacophyllum* regularly pair with bivalents from the unreduced egg of common Dallisgrass and that such pairing accounts for the quadrivalent associations in the hybrid. No doubt, some of the other *P. malacophyllum* chromosomes tend to pair with other Dallisgrass bivalents or univalents and the pairing accounts for the presence of trivalents in the hybrid. The homology of the chromosomes of the parents indicates that the species are probably related and suggests that *P. malacophyllum* could be an ancestor of common Dallisgrass.

Discussion

The chromosome number of the F_1 hybrid ($2n = 70$) suggests that it developed from the fertilization of an unreduced egg of common Dallisgrass. The regular occurrence of quadrivalents at diakinesis and metaphase I provides further evidence for this origin of the hybrid. An unreduced egg of common Dallisgrass would contain 20 bivalents and 10 univalents. Since *P. malacophyllum* has a high percentage of quadrivalents, the assumption of partial homology of the chromosomes of the parents would explain the multivalent associations of the hybrid. Apparently some of the Dallisgrass bivalents regularly pair with two chromosomes from the *P. malacophyllum* quadrivalents. Since it has been shown that the common Dallisgrass reproduced by apospory[2], there seems little doubt that the hybrid was developed from the fertilization of an unreduced egg.

The factors favoring sexual processes appear to be dominant. This work strongly indicates ance of the plants indicate that the F_1 reproduced sexually. This is particularly interesting in view of the fact that common Dallisgrass normally reproduces apomictically. Apparently the male parent was effective in restoring sexuality in the F_1 even though it contributed only 20 of the 70 chromosomes.

The factors favoring sexual processes appear to be dominant. This work strongly indicates that the balance of the factors controlling apomixis has been broken by using the one apparently sexual parent. This has been shown with sexual and apomictic hybrids of other plants[6, 8]. Breaking the apomictic barrier and restoring sexuality could result in the incorporation of desirable characters into a new and valuable pasture plant.

Thus far the method of reproduction in the F_2 has not been determined. Further cytogenetic investigation of these plants should clarify this point as well as provide interesting information on the inheritance of apomixis.

Summary

A species hybrid was obtained between *P. dilatatum* ($2n = 50$) and *P. malacophyllum* ($2n = 40$) by means of a crossing techniqse

TABLE II. Morphological characteristics of three F_2 plants resulting from a cross between *P. dilatatum* and *P. malacophyllum*

Character	Plant 1431	Plant 1432	Plant 1433
Culms	from decumbent base, nodes prominent	semi-erect, prominent nodes which are pubescent	erect, prominent nodes which are purplish
Blade	glabrous, conspicuous tufts of hair in ligule area and along edge for 1 cm., base pubescent, 8-12 mm. wide to 30 cm. long	conspicuous tufts of hair in ligule area, 6-12 mm. wide to 30 cm. long	glabrous, tinged with purple at base, leaf margins crinkled, 15-30 mm. wide to 40 cm. long
Ligule	membrane rounded, 4 mm. tall, slightly mottled	membrane obtuse, 4 mm. tall, entire	membrane mottled, 7 mm. tall
Collar	glabrous, narrow, continuous, purple at edges	glabrous, narrow, continuous	pilose on top, glabrous on bottom, margin purple
Sheath	compressed, keeled, margins tightly overlapping and red along margin	pilose along loosely overlapping margins	overlapping at base, opening 3 cm. from node, pubescent along margin
Inflorescence	8-12 racemes, spikelet 2 × 4 mm., raceme 9 cm., conspicuous tufts of hair at base of each raceme	4-14 racemes, spikelet 1 × 2.5 mm., raceme 7 cm.	15-20 racemes, sometimes paired spikelets 1.5 × 3 mm., raceme to 16 cm.
Anther color	yellow	purple	purple, yellow along suture between pollen sacs
Ergot reaction	immune	immune	highly resistant

involving proper temperature control. The chromosome number of the F_1 was found to be $2n = 70$, indicating that it was obtained by the fertilization of an unreduced egg. The appearance of the F_2 progeny and their aneuploid nature ($2n = 52$, 54, and 65) indicate that the F_1 reproduces sexually.

The male parent, *P. malacophyllum*, which is apparently sexual, was effective in restoring sexuality in the F_1. The chromosomes contributed by the sexual male parent would indicate that the factors controlling sexual processes appear to be dominant.

The results of this study show the possibility of improving apomictic Dallisgrass by hybridizing with a sexual species. The transfer of an economic character, ergot resistance, has been demonstrated.

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PHENYLKETONURIA: FIVE AFFECTED MEMBERS IN ONE SIBSHIP

F. J. KING AND BARBARA H. BOWMAN*

PHENYLKETONURIA is an inherited trait which results from the presence of two autosomal recessive genes[7]. This inborn error of metabolism prevents the conversion of phenylalanine to tyrosine because of the absence or inactivity of the enzyme which is intimately involved in the phenylalanine hydroxylation reaction[9]. The most obvious characteristic in phenylketonuric subjects is severe mental retardation. Biochemically, the affected subjects differ from non-affected individuals by having abnormally high levels of urinary and blood phenylalanine and by having phenylpyruvic acid in their urine[4, 7]. Dilution in pigment is usually encountered in phenylketonuria[3], although there have been a few cases in which the subjects have dark hair and eyes.

Penrose[11] gave evidence which indicated that the known heterozygotes for phenylketonuria tended to develop involutional psychoses. This, however, was not confirmed by the study of Jervis[8] and Monro[10].

Success in detecting the carrier of phenylketonuria as well as the development of a beneficial dietary regime for phenylketonuric patients has been accomplished in the last five years. By means of a phenylalanine tolerance test devised by Hsia *et al.*[5] it is now possible to detect the heterozygotes for phenylketonuria. A dietary regime deficient or low in phenylalanine has been studied[1] which has been shown to be beneficial to mental performance in numerous cases of phenylketonuria. The benefits of this special diet are demonstrated especially in the cases of very young phenylketonuric patients[6].

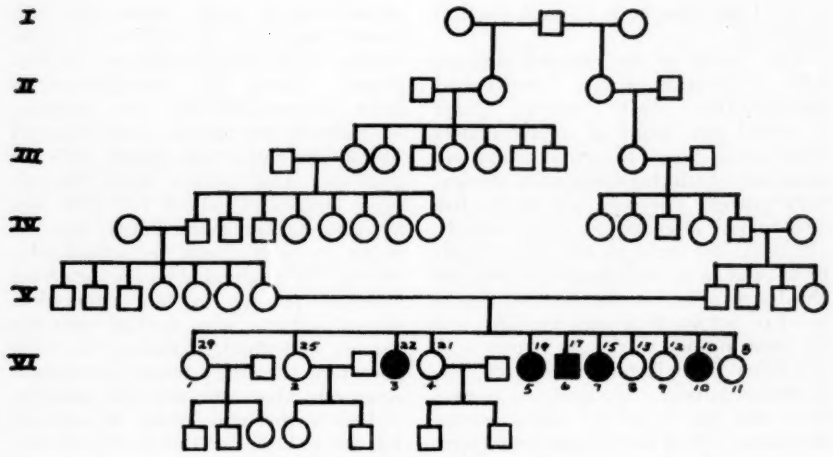
Family History

The family group described here consists of the parents and 11 children, 10 of whom are girls. Five of the children are phenylketonurics and the remaining six are unaffected individuals. Relevant parts of the family history are given in the pedigree chart (Figure 12). Both parents are in their late forties, and the ages of the children range from eight to 29 years. Three of the daughters are married; there are seven grandchildren. Among the 11 children in generation VI, the syndrome of phenylketonuria occurs in a random pattern insofar as age is concerned. In order of birth, members 3, 5, 6, 7 and 10 in generation VI are afflicted.

As far as the present investigators have been able to discover, there has been no other occurrence of phenylketonuria in the kinship for seven generations. One of the most significant facts emerging from studies of the family history is that the parents had a common great-great grandfather, who married twice. One daughter by one wife was the great grandmother of the father of the children; one daughter by the second wife was the great grandmother of the mother of the afflicted children. It is probable that the common ancestor was heterozygous for the gene pair and that he transmitted the recessive gene to at least two of his children who are ancestors of the phenylketonuric children. One cannot, of course, rule out the possibility of a mutation in one of the ancestral lines, but this assumption seems unnecessary with the available history.

The oldest phenylketonuric sib, VI-3,

*Department of Educational Psychology and Department of Zoology, University of Texas, Austin, Texas, respectively. This study was supported in part by a grant from Welch Foundation to Drs. C. P. Oliver and R. P. Wagner and in part by a grant from the National Institutes of Health. The authors wish to express their thanks to Dr. Oliver and Dr. Wagner for their helpful suggestions during this study.



PHENYLKETONURIA PEDIGREE

Figure 12

Pedigree of sibship with five phenylketonuric and six non-affected members.

is 22 years of age. Administration of the Revised Stanford-Binet Scale, Form L, yielded a mental age score of four years. Friendly and responsive, she can follow simple directions quite well and is able to do routine housework such as washing dishes. She can dress herself and is toilet trained. She has only a limited use of words and also has a rather severe speech defect. She is the only phenylketonuric sib not now institutionalized.

The next oldest affected sib, VI-5, is 19 years of age and is in good physical health. Her approximate mental age, obtained through administration of the Revised Stanford-Binet Scale, Form L, is three years. She is very docile, can dress herself, and is completely toilet trained. Verbal communication is almost absent even though she can repeat words, and she also has a severe speech defect. A marked tremor of the hands is noticeable. Physical examination showed abnormally high patellar and achilles reflexes and a positive Babinsky reflex on the right; other reflexes tested were normal.*

The only boy in the family, VI-6, is 17 years old. His approximate mental age is two years, this score having been obtained through administration of the Vineland Social Maturity Scale. Although the boy can say a few words, he ordinarily cannot make his wants known verbally. He smiles much of the time and grunts a great deal. He can dress himself and is toilet trained. While he lived at home, he occasionally had severe temper tantrums. A muscular tremor is present, and when he is sitting or standing he is restless and rocks back and forth almost continuously. Reflexes found to be hypoactive were the biceps, triceps, cremasteric, and left patellar. A right wrist and ankle clonus were found by the physician, and the right patellar reflex was hyperactive. He is spastic on his left side. He has been diagnosed as having a chronic brain syndrome as well as being phenylketonuric. The mother reported that the son had epileptic seizures in his sleep from the time he was one year of age until he reached the age

*Information concerning physical examinations was made available by Dr. Marjorie G. Lawlis, Staff Physician of the Austin State School, Austin, Texas.

of 12. Otherwise he is in good physical condition.

The fourth of the affected siblings, VI-7, is 15 years of age. The Revised Stanford-Binet Scale, Form L, yielded a mental age score of approximately three years. She is reported to have had some sort of fainting spells when she was very young. The only one of the five ever to have attended the public schools, she could not make an adequate adjustment and was withdrawn during her third year. She can dress herself, make most of her wants known verbally, and do some routine work. As is true with the other phenylketonuric subjects, articulation is poor. She grimaces a great deal and has occasional slight temper tantrums. All of her reflexes tested were normal, and she is otherwise in good physical condition.

The youngest phenylketonuric girl, VI-10, is 10 years old. Her approximate mental age is two years, this score having been obtained through administration of the Vineland Social Maturity Scale. She has no speech, but makes gutteral sounds and quick sharp cries. She grins and grimaces almost all of the time. Restlessness and occasional temper tantrums are characteristic of her behavior. Recently she has been diagnosed as tubercular, but otherwise she has no physical defects, and all of her reflexes tested were normal. The I.Q. scores of these subjects appear in Table I.

The family has risen considerably in socio-economic status within the past several years. Both the father and the mother of the affected children are employed. Before the phenylketonurics were institutionalized, the working schedules of the parents were arranged so that one parent was usually with the children. The mother went through seven grades of school (all that was available in her community), but the father received almost no formal education. Although no objective data were collected relative to this impression, several persons who worked with the parents and children during the early phases of the study made spontaneous comments about the extreme passivity of both mother and father. It is also of interest to note that the intelligence test scores (Table I) on the parents would classify the mother as normal and the father as dull normal. Whether or not the low I.Q. score of the father was markedly influenced by his lack of formal education is open to question. One normal daughter, VI-4, is known to have graduated from high school, while the normal sibs VI-1 and VI-2 finished the tenth and eleventh grades respectively. The other three normal sibs are attending public schools.

Both parents and normal sibs seem in every way to accept the phenylketonuric children in the family. The mother reported that she had wondered at times what made part of her children different from the others. She had no understand-

TABLE I. I.Q. scores and phenylalanine and tyrosine levels in serum of members of family studied

Member	I.Q.	Genotype	Phenylalanine mg./100ml.	Tyrosine mg./100ml.
V-1	100*	Heterozygote	3.2	2.0
V-2	83*	Heterozygote	1.1	0.8
VI-3	25†	Homozygote	52.6	1.8
VI-5	18†	Homozygote	39.0	0.8
VI-6	12‡	Homozygote	51.4	1.8
VI-7	21†	Homozygote	43.6	2.0
VI-8	81**	Unknown	2.3	0.8
VI-9	89**	Unknown	1.6	not tested
VI-10	20‡	Homozygote	49.4	1.1
VI-11	93**	Unknown	0.75	not tested

*Wechsler Bellevue Intelligence Scale.
**Wechsler Bellevue Intelligence Scale for Children.
†Revised Stanford-Binet Scale, Form L.
‡Vineland Social Maturity Scale (I.Q. Estimated).

ing about hereditary factors for the defect and had never heard of a similar difficulty in either her own or her husband's family. There was a complete absence of apology or explanation about the afflicted ones, and they were allowed to move at will through the house and yard. In a new neighborhood, though, there was some complaint about the children. This complication and the fact that physical growth of the children made control at home difficult led to the institutionalization of the four youngest affected children.

Biochemical Studies

Biochemical analyses of serum phenylalanine and tyrosine and the urinary amino acids were performed on blood and urine specimens from as many of the members of the sibship as could be contacted. In blood samples examined, the quantities of phenylalanine and tyrosine were determined by means of a bacterial assay as has been described by Steel[13]. The results of these analyses appear in Table I. The phenylalanine levels of the phenylketonuric sibs show striking contrast to the levels of the parents and unaffected sibs. The range among the phenylketonurics is 39-52.6 mg./100ml. of serum. The phenylalanine level of the father, a known heterozygote for phenylketonuria, is not significantly higher than the serum phenylalanine levels of unaffected individuals, i.e., 0.8-1.9 mg./100ml.[14]; however, the level in one serum specimen from the mother of the phenylketonuric children was 3.2 mg./100ml., which is appreciably greater than the serum levels of phenylalanine in the group studied by Stein and Moore[14].

The serum tyrosine levels of some of the members of the family described here appear in Table I. No significant differences in tyrosine levels of the blood of the five phenylketonuric sibs and the blood of the non-affected members of the family occurred. Tyrosine levels in this sibship are comparable to those found in 16 non-phenylketonurics, i.e., 0.9-2.1 mg./100ml.

As expected, phenylpyruvic acid was found to be present in the urine of all the affected members of the family, the levels ranging from 0.7-1.4 mg./ml. The urine specimens of the unaffected members of the family who could be contacted contained no detectable amounts of phenylpyruvic acid. Urinary amino acids occurring in the normal and affected members of the family were determined by paper chromatography[2]. Urinary levels of alanine, phenylalanine, glutamine, tyrosine, lysine, and cystine were determined in the specimens of the normal and affected members of the family; there was no abnormality detected in the concentrations of these amino acids outside of the abnormally high concentrations of urinary phenylalanine occurring in the specimens of the affected siblings.

The serum proteins of the subjects studied were analyzed by means of electrophoresis[2]. The results of the electrophoretic analyses appear in Table II. Because of the small number of subjects involved in the study, a non-parametric statistic, the Mann Whitney U-Test[12], was considered to be the most appropriate in making tests of the significance of differences between the normal ($N = 5$) and the abnormal ($N = 5$) groups. Two of the five blood measures listed in Table II, alpha I globulin and alpha II globulin, differentiate at high levels of significance ($p. = .01$) between the two groups. While none of the alpha I and alpha II globulin values for the abnormal group deviate markedly from normal values, it may be seen that no overlap between the groups occurs. Beta globulin tends to differentiate between the groups, but the overlapping values for one normal sibling (VI-11) and one abnormal sibling (VI-10) keep the difference from being significant.

Summary

A sibship is described in which five of the

TABLE II. Serum proteins in members of family studied*

Member	Albumin	Alpha I globulin	Alpha II globulin	Beta globulin	Gamma globulin
V-1	61.6	3.5	9.0	11.5	14.5
V-2	55.5	4.6	10.4	12.9	16.9
VI-8	56.5	4.4	9.5	13.3	16.4
VI-9	60.0	3.6	11.2	11.9	13.3
VI-11	67.5	7.5	9.0	7.2	8.4
VI-3†	65.9	3.4	6.7	9.9	14.1
VI-5†	66.3	2.3	7.3	8.5	15.7
VI-6†	68.3	3.2	8.2	9.1	11.1
VI-7†	67.8	3.2	8.3	9.8	11.1
VI-10†	60.7	3.4	7.9	13.7	14.1

*Values reported are in terms of percent of total serum protein.
†Phenylketonuric.

11 siblings are phenylketonuric. The investigation of the family's pedigree revealed the parents of the phenylketonuric children had a common great-great grandfather. The I.Q. scores of the parents, the affected siblings, and the non-affected siblings who could be contacted are given. The serum levels of phenylalanine and tyrosine were found for this group. The serum phenylalanine level in one parent appeared to be elevated compared to the reported levels of unaffected individuals, although the level in the other parent appeared to be normal. There was no difference observed in the tyrosine levels in the serum of the phenylketonuric children in comparison to the levels in a group of non-affected individuals tested. There was a strikingly high concentration of phenylalanine in the serum of each of the affected members of the sibship as well as high levels of phenylpyruvic acid in their urine samples. Significant differences for two serum proteins were observed between affected and non-affected members of the family. Paper chromatography revealed no abnormality in the concentration of the urinary amino acids in the parents or in any of the siblings of the sibship.

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WHY HUMAN GENETICS?

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THE literature of genetics is replete with instances of "unit characters" which are under the control of single pairs of alleles, one dominant, the other recessive. As long as one is concerned with characters which fall approximately into this category, the situation even in the human field remains relatively simple and prospects of smooth sailing bright. The farther one departs from this ideal situation, however, the more uncertain is the course. Hence there is the temptation to direct one's attention predominantly to those genetic items for which it is possible to discover rather simple interpretations and thus to minimize attention to items which may be of greater importance in the broad realm of human affairs.

The basis for my interest in human genetics rests upon characters which are certainly not under the simple control of single gene pairs but which nonetheless have a genic origin. Besides mutations, such phenomena as incomplete dominance, complete lack of dominance, gene interaction of many kinds, recombinations, ploidy, chromosome aberrations and cytoplasmic effects, are widespread and serve to complicate any study which seeks to elucidate precise mechanisms. The single gene effects are sufficiently obscured so that, to quote David and Snyder[5], "it has become more and more widely recognized that single-gene differences which produce readily distinguishable discontinuities in phenotypic variation are completely *non*-representative of the bulk of genetic variability in any species."

Before pursuing this subject further, it will be well to bring into clear focus the considerations which are basic to the interest in human genetics with which the writer is concerned. There are, it appears, three distinct objectives which one might have in pursuing a study of human genetics: (1) one may be satisfying scientific curiosity—developing a pure science; (2) one may be concerned with gathering information that will find application in improving the human race; (3) one may be interested, for many practical reasons, in a better understanding of the human race, regardless of any eugenic possibilities.

It would not be possible for me to argue against the first objective, the development of pure science. It is possible, however, to take the position that the use of human material in the development of a science of genetics is of debatable importance. The second objective, eugenics, does not engender maximum enthusiasm among the best informed because the means of reaching the objectives are relatively uncertain and the results which can reasonably be expected are by no means revolutionary. The third objective, that of understanding the people who constitute the human race, has certainly not been clearly outlined and emphasized by geneticists in the past. It is *by far* the most important, in the writer's opinion.

There are ample, broad and extremely diverse reasons for thinking that a knowledge of human heredity can contribute enormously and in many ways to the understanding of the human beings with whom we deal continuously. Human genetics has potentialities for contributing to the understanding of the people not only from the standpoint of the diseases that afflict them, but also from the standpoint of their temperaments, their mental operations, their emotions, their motivations and drives, their diverse aptitudes and abilities, all of which greatly affect their functioning as members of society. This may be a somewhat long-range objective but not as long-range, I believe, as one might suppose at first glance. It seems obvi-

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ous that this objective cannot be attained by increased attention to the discovery of new genes, the determination of gene frequencies, or mutation rates, or the intensive development of population genetics. Except for the last item, these are concerned primarily with single gene differences which are not representative of the kind of genetic variability which is of the greatest moment to the human race.

While it is true that complexities can be best mastered if the elementary principles are first thoroughly elucidated, it is too much like putting human genetics in a strait jacket to insist that only the simpler items in human heredity—single gene differences—can profitably be studied. Biological science, for example, has made progress in many areas where the underlying chemical processes are obscure. In a parallel fashion human genetics should be able to advance and contribute knowledge about heredity in areas where the details of the underlying mode of inheritance are likely to remain somewhat foggy for decades to come. For clearing up the basic principles which apply to human genetics, the use of direct human study is most cumbersome.

There has been a detectable tendency on the part of some geneticists to bring at least into their semi-popular writings the implication that characters, the inheritance of which does not fall within the purview of relatively simple genetics, are largely environmentally induced. This implication needs careful scrutiny. The joint statement[15] made by a group of the leading geneticists—Muller, Dobzhansky, Demerec, Levine, Schrader and Snyder—is worthy of consideration at this point.

"At the present level of genetic knowledge we cannot improve the genes with which we are born, and there is little we can do to determine which genes we pass on to our children. But we can improve the manifestations of our own genes and those of other persons so that the best possible phenotypes are produced. The control of the manifestation of human genes is, of course, possible only by proper manipulation of the environment. The implications as regards medicine, especially preventive medicine, are obvious. As Dobzhansky of Columbia University has put it: 'Medicine is the science of management of the human phenotype.' "

This statement is obviously for the consumption of the general public—not for fellow geneticists—and as such fails to convey adequately to people who probably know little about genetics, except perhaps of the existence of dominant and recessive genes, the important fact that a tremendous array of *interacting* genes are involved in human heredity and the single genes "with which we are born" and the single genes "we pass on to our children" constitute only a very minor part of the whole complex story of human heredity. I believe non-geneticists reading this statement would be led to conclude, in terms of their own limited knowledge of the subject, that the variability which does not come under the play of dominant and recessive genes is probably environmentally induced.

This statement also makes it plain that medicine, particularly preventive medicine, is the one outstanding potential application of genetics to human affairs. To the writer it seems clear that numerous and pressing human problems quite aside from those of a medical nature are also amenable to a "proper manipulation of the environment."

An important point which may have been implied in the above statement, but should have been made explicit for the benefit of the readers to whom it was directed (non-geneticists), is that knowledge about the potentialities of individual genotypes (each individually distinctive) is *essential*, so that the "proper manipulation of the environment" can produce "the best possible phenotypes." This seems to be the most important objective of human genetics—understanding the potentiality of individual genotypes (encompassing an exceedingly complex assortment of interacting genes) so that the environment can be manipulated intelligently and effectively.

Many social scientists who welcome such a statement as that quoted above extend its meaning to include the idea that an environment can be devised which, when applied generally to the human race, will yield the best phenotypes. Strictly from the genetic standpoint, nothing could be farther from the truth. Each genotype might be expected *a priori* to require for best results a distinctive environment patterned after its own needs. What human genetics can contribute is the vast amount of knowledge about genotypes which will make possible the intelligent manipulation of the specific environments to bring about the desired ends whatever they may be.

My interest in these questions has been stimulated by the finding in our laboratory that inbred rats, supposedly of uniform stock, differ widely in their urinary excretion patterns[12] and in their tendencies to consume alcohol in choice experiments[13], when kept side by side on identical diets. Furthermore, these observed variations are diminished but by no means abolished when the animals tested were closely inbred specimens (said to be brother-sister mated for as many as 101 generations). Among four rats belonging in this category[12], there were seven instances in which, when using seven to 11 urinary samples from each rat and testing for nine amino acids, differences in the excretion of individual amino acids differed consistently by 200 to 300 percent. In one instance one of these rats (#13) excreted over 10 times as much phosphate *in the urine* as did another (#3) although they were both eating exactly the same food.

Inbreeding should eventually produce a stock which is *relatively* homogeneous. When we take into consideration, however, all of the complications which are known to enter, including mutations, recombinations, occasional chromosome aberrations, accompanied by the complexities of gene interaction and cytoplasmic influences (as well as possible mistakes and unavoidable or unconscious selection of heterozygotes during the breeding program), then we might expect to arrive at a stock which is very far from homogeneous genetically. The production of such a heterogeneous stock even in spite of inbreeding is what our observations suggest.

An older study[3] has also come to my attention which points even more conclusively in the same direction. These authors found among 645 male rabbits of the same breed that 17 organ weights, corrected for total body weight, showed on the average a 14-fold variation, with a median variation of 10-fold! That these are environmentally induced differences is difficult to square with the known facts regarding the inheritance of external morphological traits. Another study made by Rosahn and Casey[14] indicates that among rabbits of the same strain (and also incidentally in normal humans), there are persistent wide interindividual variations in blood morphology. A beginning has been made in our laboratory of similar studies involving the organ weights of inbred rats, mice and baby chicks, and also the blood morphology of chicks. The findings indicate these animals (which are certainly far more inbred than a typical human population would be) are genetically heterogeneous in the same way.

It seems that these observations are of great importance so far as their human implications are concerned because obviously human beings also show wide variations in the same way that animals do. A more adequate understanding of the roots of this wide variability is needed. There are other possible explanations for the wide variability of "inbred" strains besides those of mutations and the possibility that "inbred" animals may not be as completely inbred as supposed. There are complexities of gene interaction for which inbreeding may not be able to compensate, as well as various types of cytoplasmic influences which are not contributed equally by the two parents and obviously do not follow Mendelian rules.

The moment one admits that extragenic (cytoplasmic) factors play a role in mammalian inheritance (and many leading geneticists accept this as a fact),

then the possibility exists that in the case of characters for which the mode of inheritance is undetermined, the extra-genic factors are not of trifling but of *major* importance. This concept is not in conflict with the truly marvelous developments with respect to the functioning of genes in heredity, but it calls attention to the possibility that for characters not yet investigated, extra-genic influences (co-influences with genes, presumably) are important.

In mammalian and fowl genetics the possibility of the effect of cytoplasmic influences is indicated by the results of reciprocal crosses between horses and asses (mules and hinnies) and between different types of pheasants[10, 11]. The older literature differentiates between mules and hinnies as two distinct groups; Webster's dictionary is very explicit as to their differences. The sex ratio among hinnies is reported to be 58 percent females and 42 percent males while that of mules is 39 percent females and 61 percent males[4]. In the popular literature there are references to reciprocal crosses between lions and tigers (tiglions and ligers) but these appear not to have been investigated scientifically.

One of the well-known facts which emphasizes the existence of vast "unknowns" in the genetic field is that the cellular genetics involved in the processes of differentiation is poorly understood. During this process the chromosomes in differentiated cells show the same bands, but they markedly differ in the presence of "puffs" and "bulbs," indicating that changes involving genes have taken place[2]. Since it seems highly probable that the interaction between genes and cytoplasm is crucially important during differentiations, and if so, who can say that cytoplasmic influence is not important in determining such large and unequivocal differences as those observed in organ sizes in inbred strains?

Let us assume for purposes of discussion that each egg is distinctive both as to genes and as to cytoplasm and that each sperm cell is distinctive in the same way. (The amount of cytoplasm in sperm cells is not large but no one can be sure that it lacks significance.) After long continued close inbreeding (barring complications) the genic make-up of the fertilized eggs may become uniformized. The situation with respect to the cytoplasmic, extragenic material is different, however, and there is no assurance that it is uniformized in the same way. This constitutes one explanation of why inbreeding does not produce uniform animals. Whether it would be possible by any means (such as mating a single father with many generations of daughters, granddaughters, great-granddaughters, etc.) to produce more uniform progeny (with respect to organ weights, for example) is not known.

The problem of monozygotic twins naturally comes into this discussion. It is interesting that Kallmann[8] states, "Discordance between them is not, as is commonly assumed, a measure merely of postnatal or even of prenatal development; it may also have a genetic component." Differences in prenatal or postnatal environment probably could not explain such extraordinary cases as that reported by Warkany *et al.*[18] in which at the age of eight one monozygotic twin became obese and the other a diabetic.

Many one-egg twins show such strong resemblances that the term identical has often been applied. These strong resemblances may be based not only upon the identity of their genes but on the fact that the cytoplasmic influences are also *similar*. There is no assurance, however, that when the first cell division takes place, the cytoplasmic material of the zygote (derived from both the egg and the sperm cell) is distributed exactly equally, quantitatively and qualitatively, between the two daughter cells. In fact evidence is to the contrary. Ordinarily it would appear that these two cells are *about* the same and have *about* the same potentialities. In our study of the excretion patterns of one pair of monozygotic twins, we found *strong* resemblance, but not identity[19]. The fact that in some cases monozygotic twins have two separate placenta[17] and in others only one (as well as other structural

differences) may not be wholly accidental; these may be associated with differences in the distribution of cytoplasmic material between the first two daughter cells.

Another possible explanation of the wide variation in the organ weights, etc. of highly inbred animals lies in the complexities of the interaction between genes. While inbreeding tends to establish a definite gene pool which is passed on to the offspring, there appears to be no assurance that recombinations might not cause the animals to be heterogeneous because of differences in interactions between the existing genes. In other words, what determines the characters in an animal is not only "the genes with which they are born" but the important variations in the potential interactions of these genes.

Still another possible explanation of the widely different patterns of organ weights possessed by inbred animals is somatic mutations. These mutations would have to be very common to produce the effects which have been observed, and for various reasons it seems that this explanation is only possible, but not probable.

Regardless of what interpretation we may accept with respect to the variability in "inbred" strains of animals, a consideration of the facts is highly pertinent in the realm of human genetics because so-called normal human beings exhibit similar enormous variability. Endocrine glands, for example, vary in size and activity over wide ranges—often 10-fold or more[20]. Wide variation is the general rule in the realm of composition, enzyme activities, excretion patterns, pharmacological reactions, and nutritional needs, as well as in miscellaneous physiological areas. Furthermore these diversities exist so far as we know within every ethnic group and hence are universal.

In the field of medicine I do not believe that genetic factors are considered as seriously as they should be, and that geneticists are partially to blame. One geneticist, for example, refers to "a patient with a genetic condition." Reference was probably to a patient with hemophilia or some well-established hereditary disease, but the phrase as used suggests that some diseases have no genetic roots. This, in my opinion, is clearly not the case[21, 22]. In the strict sense no patient ever went to see a physician who did not have to greater or lesser degree "a genetic condition." Ample evidence indicates that even susceptibilities to infectious diseases are inherited, though of course the exact modes of inheritance are not known.

Among the more interesting anatomical differences found in human beings are those present in the brain. Lashley[9] has said, "The brain is extremely variable in every character that has been subjected to measurement. Its diversities of structure within the species are of the same general character as are the differences between related species or even between orders of animals . . . individuals start life with brains differing enormously in structure, unlike in number, size and arrangement of neurons as well as in grosser features." He might have added that the number of neurons does not change during later processes of development.

No one is in a position to deny the importance of the "enormous" existing differences in the brain structures of "normal" people. Concerning the inheritance of such anatomical features we are in complete ignorance and are likely to remain so unless more geneticists are induced to tackle problems of this character.

The functioning of human minds is without doubt closely correlated with brain anatomy and function. We know that a multitude of human troubles, including the devastating ever-present threat of war, start in men's minds. What could be more logical than that biological scientists be interested in human brains and whatever genetic factors enter into their make-up?

We need desperately to understand men's minds better than we do, and on the basis of differences in brain structure alone there is ample reason for thinking that inheritance enters into the

quality of everyone's mind.

Yet the most influential school of thought in psychology and particularly psychiatry is one which traces the vagaries of individual minds back to environmental influences during infancy or even in some cases to prenatal life. Walter C. Alvarez[1], the veteran physician, complains about the lack of attention to heredity in this field: "in most of the present day books on psychiatry, there is not even a short section on heredity. The book resembles a text on paleontology written for a fundamentalist college, with not one word on evolution."

By implication differences in brain anatomy are of no consequence, and human geneticists appear to acquiesce in the assumption that mental traits are environmentally induced. In spite of the abysmal ignorance which surrounds the subject of how minds differ, a UNESCO publication, inspired by the current attitude of some human geneticists, says: "Such biological differences as exist between members of different ethnic groups have no relevance to problems of social and political organization, moral life and communication between human beings." The excuse for such a statement rests upon the fact that unquestionably there is tremendous diversity *within* each ethnic group. But to say or imply that the diversity of the working of men's minds (whether within a relatively homogeneous group or in humanity at large) is inconsequential (and presumably environmental in origin) is stepping far beyond anyone's knowledge and against strong biological evidence.

Most of the genetic studies which have dealt with "intelligence" have been painfully inadequate, largely because the studies have not been truly interdisciplinary in the sense of involving top experts in both psychology and genetics. Most studies have been concerned with "I.Q." tests.

The so-called "intelligence quotient" of a child is determined by applying a series of supposedly comprehensive tests involving mental functioning, and then averaging the results obtained. There are complex arithmetical, verbal, spatial, memory and speed factors in the tests and the final score is a summation of everything that goes into the test. The question "Are intelligence quotients inherited?" is, in view of these facts, not a very appropriate or at least not a very penetrating one.

Thurstone in his extensive work has found strong evidence that "mental ability" is made of a number of separate abilities which are by no means always highly correlated, and Guilford[7] has recently indicated that the number of facets to human minds is not less than 40.

In principle, dealing with "intelligence" as though it were a single trait the inheritance of which could be studied in a definitive manner is as questionable as it would be to study the inheritance of "anatomy" as a single trait composed of skull volume plus interpupilary distance plus length of fingers plus size of gonads plus a number of other distantly related measurements all lumped together to form an "anatomical quotient."

Thurstone[16], using twin studies, obtained evidence that the patterns of men's minds (made up of their primary mental abilities) are inherited, and this might be expected, but it seems very dubious indeed whether any worthwhile data relating intelligence to genetics could be obtained as long as intelligence is considered as a single character.

One very important component of intelligence is creativity. It is generally if not universally admitted that no method for testing this is known. It is also widely appreciated that creativity does not correlate highly with the kind of intelligence which resides in highly proficient "lesson getters." Recently Getzels and Jackson[6] have gained direct evidence on this point. They compared two groups of selected youngsters, one with IQ's averaging 150, another with IQ's averaging 128. The group with the lower IQ's were judged to have more originality, sparkle and independence of thought than those with the very high IQ's.

The idea that differences in people's minds are induced solely by environmental and developmental influences is

reflected in the concept of mental age. If a child does not exhibit a high intelligence quotient, it is said to be because he is not "old" enough mentally—all he needs is time and a suitable environment for development. There is a strong trend in recent years to substitute the expression "retarded child" for a "mentally deficient child." This may be a more humane designation but it should not carry the implication that mental deficiencies are environmental and developmental in origin unless the implication is justified by the facts.

Actually one of the strong reasons for recognizing "patterns of mind" rests upon the fact that individuals who, according to current tests, are "mentally deficient" may have special abilities that are really "superior" even in comparison with the general population. Individuals who exhibit special abilities far out of line with the usual tests are to be found in every institution where feebleminded children are cared for.

From the humanitarian and democratic point of view, it seems far better to recognize many patterns of mind, and cease to rate people in categories of superiority and inferiority. Voters include "very superior" individuals (I.Q. 130 to 139) and "dull" individuals (I.Q. 80 to 90) and we count all the votes equally. This would not make sense if the adjectives "very superior" and "dull" were correctly applied. Actually the existence of "patterns of mind" makes it possible for a "dull" person to have better political judgment than one who is classified in general as "very superior."

From the standpoint of understanding human minds it is crucially important that we recognize the existence of mental patterns and learn more about them and the genetic factors which must enter into their production.

One of the reasons why some geneticists may not care to entertain ideas such as we have expressed and hence may not care to further the objectives which have been outlined in this discussion, is their violent reaction against the terrible doctrine of racism. They fear that careful attention to patterns of mind and their inheritance will result in discovering that different races do exhibit significantly different patterns—a finding which they feel would feed the fires of racism.

The writer has a profound and scientifically respectable faith that we may safely follow the truth in all areas wherever it may lead. In this particular area of innate racial differences he believes that a search for understanding will do far more in the end to cultivate interracial good will than any other line of endeavor. One of the important findings that is bound to come from such investigation is that extreme diversity of mind exists *within every ethnic group*. This finding will serve as a scientifically potent denial of the idea that individuals from different ethnic groups can be "stereotyped," and will furnish a real and convincing basis for the concept that every individual of whatever racial origin is entitled to be judged as an individual on his own merits.

From the standpoint of manipulating the environment in the area of education it is of the utmost importance that the true nature of human minds be understood. To what extent can we make the educational environment uniform (rigid requirements) and when must we manipulate the environment by giving certain students what they peculiarly need? The only basis for answering this question is knowledge about human genotypes—knowledge that only human geneticists can furnish.

Within reasonable space it has been possible to discuss only a few illustrative phases of a very complex subject. We need to know far more than we do about the extent to which various human attributes and behavior are genotypical in origin. Do sleep patterns have predominantly an environmental origin? Is liking for warm or cold climates merely based upon previous climatic experience? Are special gustatory likes and dislikes merely conditioned by previous experience? Is love of poetry (or a particular type of poetry) induced environmentally? Are histrionic leanings

primarily the result of training? Are temperaments merely cultivated? Is creativity a natural endowment, or does it arise because of special nurture? Do emotional disorders arise primarily as a result of childhood experiences or does inheritance contribute in an important way? To what extent are special aptitutes—mechanical, mathematical, musical, etc.—environmentally induced? These are some of the many questions which only human geneticists can answer.

The most important task of human genetics is to gain understanding of the potentialities of human genotypes so as to make possible the intelligent "manipulation of the environment."

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HARELIP IN SHORTHORN CATTLE

J. D. WHEAT*

HARELIP, a congenital cleft in the upper lip, is caused by faulty fusion of the median nasal and maxillary processes. The fleshy lip or the bony upper jaw, or both, may be affected. The condition may be double, with a cleft on each side of the mid-line of the upper lip, or single with a cleft on the mid-line or on either side of the upper lip.

Harelip in humans is usually unilateral, on the left side, and is often combined with a cleft palate[1]. Expressivity varies from slight external clefts to very severe clefts of the soft, and even the hard, palate[2]. Penetrance is incomplete, but is higher in males than in females (more than 60 percent of the affected individuals are males), and more severe types of expression occur mostly in males[3].

Three of the five cases of harelip in cattle reported by Weber[4] were combined with cleft palates.

The purpose of this paper is to report the occurrence of two unilaterally harelipped white Shorthorn bull calves in the Russell S. Adams, Jr. herd near La Harpe, Kansas. Both calves had phenotypically normal parents.

HARELIPPED SHORTHORN

Figure 13

First affected animal, A, which was unilaterally harelipped on the right side.

Description

The first affected animal, born March, 1952, was unilaterally harelipped on the right side. The writer did not see this animal (shown in the feed lot, Figure 13), but according to Mr. Adams, the steer completely submerged his muzzle while drinking and displayed a peculiar scooping motion while eating from a trough. The animal's incisors had apparently decayed by the time he was placed in the feed lot, and although he exerted additional effort to attain and hold feed in his mouth, he gained weight as rapidly or perhaps more rapidly than the other animals in the lot at that time. The steer was sold and slaughtered and no thorough examination was made of the extent of the anatomical defect or damage resulting from it.

The second calf (Figure 14), born March, 1958, was examined and photographed when he was five months old. The calf was unilaterally harelipped on the left side and the dental pad on that side was missing, but the hard palate was formed. The cleft extended back about two inches, or to about two inches anterior to the first premolar tooth.

When the second calf was quite young, he was unable to consume all of his mother's milk. Consequently, another

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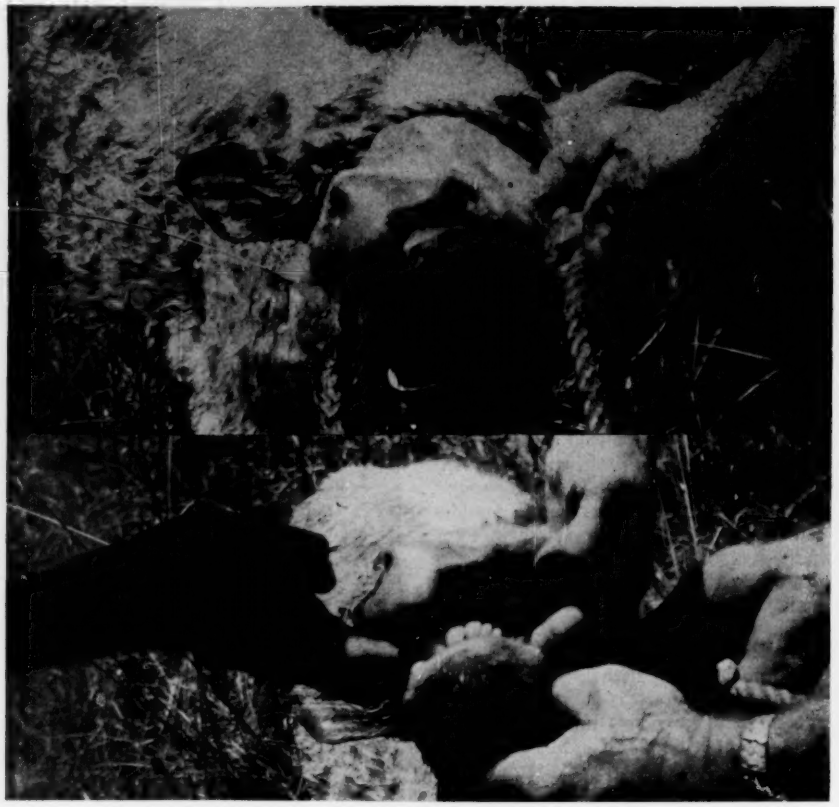


HARELIP ON THE LEFT SIDE

Figure 14

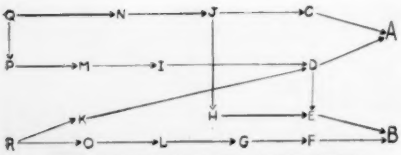
Second affected animal, B, which was unilaterally harelipped on the left side.

calf of the same age was allowed to nurse the harelipped calf's dam and the three animals ran together in the same pasture. When the calves were five months old, it was apparent that the harelipped calf had not successfully competed with the other calf for his dam's milk. It was assumed that the deformity hindered the calf's nursing ability.

Inheritance

Most of the presently known hereditary anomalies among domestic animals are caused by recessive genes. Inbreeding is expected to increase the frequency of such anomalies, but it could not have been a major factor in these Shorthorns since each affected animal was only 39/100 of one percent inbred. However, further analysis of the pedigree (Figure 15), which includes all pertinent individuals in six generations for the two affected animals, revealed they were slightly more than 16 percent related, i.e., about one-third more closely related than first cousins in a random mating population.

Stern[3] concluded that in many human pedigrees, harelip and cleft palate depend on the presence of various specific alleles at several autosomal loci and also require the presence of mostly uncontrolled environmental factors. However, Neel and Schull[2] reported that occasionally simple harelip and cleft palate (unassociated with dental anomalies other than those due to the cleft palate or congenital fistula



RELATIONSHIP OF AFFECTED ANIMALS

Figure 13

Diagrammatic pedigree showing relationship between affected animals A and B.

of the lower lip) may be caused by a simple dominant gene with high penetrance.

The fact that this developmental abnormality which rarely occurs in cattle appeared twice, in a six year period, in related animals in a herd averaging only about 30 cows, indicates it probably has a genetic basis. According to the available information, harelip in these cattle could have been caused by a simple recessive gene, by epistatic gene action, by a simple dominant gene with incomplete penetrance, or by other possible types of gene action. Since the affected animals were essentially non-inbred, but were comparatively closely related, one of the possible hypotheses appears to be that harelip is caused by a combination of epistatic gene action and undetermined environmental factors. However, on the basis of the available information, it is impossible to definitely determine the manner in which harelip in these Shorthorn cattle was inherited.

Summary

Two unilaterally harelipped Shorthorn bull calves are described. The extent of the anatomical defect and damage resulting from it were not determined for the first calf, but he was harelipped on the right side whereas the second calf was harelipped on the left side. The second calf had no dental pad on the left side and the cleft extended back about two inches or to about two inches anterior to the first premolar tooth. The hard palate was formed.

The inbreeding coefficient for each calf was only 39/100 of one percent, but they were slightly more than 16 percent related. Harelip in Shorthorns probably has a genetic basis, but the data were not adequate to make it possible to definitely determine its mode of inheritance. A possible hypothesis is that harelip is caused by epistatic gene action and undetermined environmental factors.

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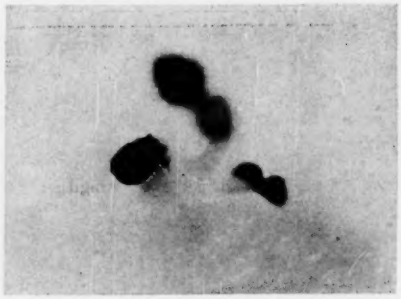
HAPLOIDY IN TOBACCO

DREUX DE NETTANCOURT AND G. W. STOKES*

HAPLOID-DIPLOID twinning has been reported in several plant species. Maheshwari[2] lists nine genera and 10 species where haploid-diploid twins were found. Production of haploid organisms and the homozygous diploids derived from them represent an ideal approach to an understanding of the interaction between genotype and environment. Because of the amphidiploid origin of *N. tabacum* L. ($n = 24$)[1], one might expect survival of the haploid plant, whereas haploidy in the more commonly thought of diploids would not be expected with equal frequency.

The study herein reported was an effort to obtain *N. tabacum* haploids through the use of techniques to be discussed.



TWIN SEEDLINGS
Figure 16
Twin seedlings arising from one seed.

Materials and Methods

Two techniques were helpful in detecting haploids. The first (twin technique) was based on polyembryony which occurs in *Nicotiana*[2] and that haploid parthenogenesis would give rise to the extra embryo. One hundred and four thousand seeds resulting from self-pollinations and representing 12 varieties of burley and dark tobaccos were sowed in petri dishes. Shortly after germination the young seedlings were searched for twins (Figure 16). The second method was based on a recessive character used as a seedling marker (recessive character technique). The hypersensitive or resistant reaction to tobacco mosaic virus (TMV) is dominant and was used as a male parent marker in crosses on the recessive mosaic-susceptible tobacco. The resulting seedlings were allowed to reach first true leaf stage and then were inoculated with TMV[4]. Susceptible plants were saved for further study. Approximately 2,500 seedlings were screened using the recessive character technique. Other recessive characters, virescence[5] and consolation[3] were used without success.

Chromosome counts were made on root tips of plants where haploidy was suspected and those confirmed were grown to maturity.

Results

The 104,000 germinated seeds gave rise to nine pairs of which 10 seedlings died. Among the survivors one individual of a surviving twin pair proved to be haploid and came from a seed of the dark tobacco variety Ky 151.

Two plants showing mosaic appeared in the F_1 Ky 16 (nn) $\times$ Ky 35 (NN) population. All plants resulting from fertilization by Ky 35 were hypersensitive and were killed following TMV inoculation, while those arising as a result of female pollen contamination or female haploid parthenogenesis were mosaic susceptible and survived. One of the two surviving plants proved to be haploid. The recessive character technique is useful when the hypersensitive TMV factor is used because selection is greatly

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HAPLOID-DIPLOID TWINS
Figure 17
The haploid (left) is slightly smaller than its diploid twin (right).

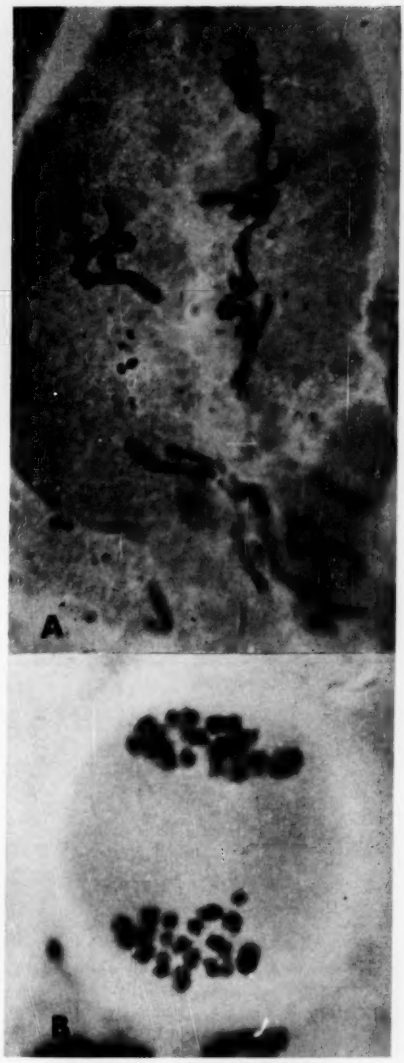
simplified. The only limitation is the presence of TMV in the haploids.

Observations and Conclusions

The haploid twin was slightly smaller and flowered eight days earlier than its diploid counterpart (Figure 17). The leaves were more narrow and the stomata were approximately two-thirds normal size. Corolla diameter, length of filaments, and size of anthers were reduced in approximately the same proportions. The few apparently normal pollen grains of the haploid measured approximately 25 microns as compared to 35 for normal tobacco.

Twenty-four chromosomes were counted in the root tip cells of the haploids (Figure 18*A*). In meiosis the number of chromosome units (bivalents + univalents) per metaphase I nucleus ranged from 19 to 22 suggesting a pairing range of two to five bivalents. It is probable, however, that some of the chromosome units observed were closely associated chromosomes without segmental homology. Bivalent-like configurations were observed in a few cells. Synapsis was possibly due to segmental homology and influenced by the position of the chromosomes in the nucleus. Chromosome numbers ranged from 14 to 22 in metaphase II, indicating that some of the univalents divided equationally in metaphase I (Figure 18*B*). The equational division of the metaphase I univalents would give rise to more fertile gametes than would otherwise be expected. This supports the data gained when the haploid was used as the female parent.

The range in fertility of the F_1 haploid $\times$ diploid excludes the assumption that only restitution nuclei were functioning since restitution nuclei would give rise to uniform gametes. Giant pollen grains and a low frequency of normal-appearing pollen grains were observed in anther smears but were probably not functional since seed did not set when the haploid



HAPLOID CHROMOSOMES
Figure 18
A—Somatic cell of a haploid with 24 chromosomes. *B*—Metaphase II in the haploid.

was used as the male parent.

Fourteen seeds that gave rise to eight seedlings were produced following six crosses (one capsule produces approximately 2,500 seeds) haploid × diploid, indicating a low level of female fertility in the haploid. Four of the F_1 seedlings were sterile and did not set seed upon selfing while the remaining four set an abundance of viable seed. Pollen grain observations in both sterile and fertile individuals revealed differences in the amount of fertile pollen. The differences in fertility of the F_1 haploid × diploid plants indicate possible differences in female gamete chromosome numbers.

Summary

Two *N. tabacum* haploids, one dark and one burley, were found during the course of this study.

The twin technique indicates that approximately one seed in 10,000 contains two embryos.

The selection of haploids by the recessive character technique was dependent upon nondeliterious recessives as indicators.

Guard cell length, corolla diameter, filament length, anther and pollen grain size were approximately two-thirds normal size in the haploid.

Equational division of metaphase I chromosomes was frequent in the haploids.

Equational division of metaphase I chromosomes increased the female fertility of the haploid.

Variation in fertility level of F_1 haploid × diploid plants indicates viability of female gametophytes of meiotic origin.

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